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

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Publication date
2016

Document Version
Final published version

Citation for published version (APA):
5. Gene-by-Intervention Interaction including The Incredible Years, COMT and MAOA: Is Reward Sensitivity a Mechanism?

Abstract

The goals of this study were: (1) to explore a previously reported Gene-by-Intervention (G×I) interaction between the effects of The Incredible Years program (IY) and a polygenic dopaminergic index consisting of enzyme-related polymorphisms (COMT, MAOA) in boys and (2) to test whether this interaction could be explained by genetic differences in reward sensitivity, specifically sensitivity to parents' use of praise. The original sample consisted of 387 Dutch children between 4 and 8 years of age (Mage = 6.31, SD = 1.33; 55.3% boys) and their parents, who participated in a three-wave randomized controlled trial. Results of completers only analyses on boys (n = 192) indicate that the previous reported polygenic G×I was specifically due to the MAOA polymorphism: The effects of IY on child externalizing problems were stronger for boys carrying the MAOA low-activity allele, compared to boys carrying the high-activity allele. Theoretically, these children might be specifically sensitive to reward due to upregulated dopamine availability. However, although our results show that IY caused a large (partial η² = .19) increase in parents' use of praise, this increase did not explain the intervention effects on child externalizing problems in boys with and without the MAOA low activity allele. This might indicate that reward sensitivity might not be a mechanism underlying G×I or that we did not fully capture reward-oriented parenting due to limitations of our observation data. An important next step in G×I research will be gaining insight into the underlying biopsychosocial mechanisms.
Individual differences in how (strongly) children are affected by their surroundings have become an important theme in research on child development (Belsky, 1997; Boyce & Ellis, 2005). There is growing evidence that the strength of the relation between children’s environment and the development of both adaptive and maladaptive behavior differs due to children’s genetic make-up, specifically dopamine related genes (i.e., gene-by-environment interactions: G×E, for overviews and meta-analyses see Bakermans-Kranenburg & Van IJzendoorn, 2011; 2015; Byrd & Manuck, 2014). Interestingly, the same genetic markers that predict disproportional negative outcomes under adverse circumstances have also been shown to predict more favorable outcomes when the environment is enriched through intervention (i.e., gene-by-intervention interactions: G×I, Van IJzendoorn & Bakermans-Kranenburg, 2015). These genotypes might therefore not just predict an increased vulnerability for environmental risk but instead predict an increased susceptibility to both environmental risk and enrichment.

Indeed, a recent meta-analysis including 22 genetically informed randomized controlled trials showed that intervention effects are stronger for individuals with “susceptibility genotypes” than for individuals without such genotypes (Van IJzendoorn & Bakermans-Kranenburg, 2015). This suggests that failure to take into account such child characteristics might lead to over- or underestimation of intervention effectiveness for individual children. In addition, incorporation of these child characteristics might increase our insight into “what works for whom” and, in turn, enable us to tailor interventions accordingly to increase their effectiveness. In a previous paper we showed that in boys the intervention effect of the behavioral parent training (BPT) The Incredible Years (IY) was moderated by a dopaminergic polygenic factor (i.e., “plasticity index”) consisting of receptor- (DRD2, DRD4), transporter- (DAT1), and enzyme- (COMT, MAOA) related polymorphisms (Chhangur et al., in press). Further investigation showed this G×I effect was specifically due to variability in polymorphisms coding for the enzymes catechol-O-methyltransferase (COMT) and monoamine oxidase A (MAOA) (Chhangur et al., submitted for publication), which are responsible for degradation of amines among which the neurotransmitters dopamine. Specifically, boys in the intervention condition carrying more enzyme-related “plasticity alleles”, namely the low-activity alleles of the MAOA gene and/or Val-alleles of the COMT gene, showed a significantly larger decrease in externalizing behavior compared to boys in the intervention condition who carried fewer of these alleles.

The COMT Val-allele is related to more catechol-O-methyltransferase production, leading to faster inactivation of dopamine and lower dopamine availability (Chen et al., 2004). In contrast, the MAOA low-activity allele is related to less monoamine oxidase A production, resulting in less degradation of dopamine and higher dopamine availability (Weyler, Hsu, & Breakafield, 1990). Our G×I finding that IY works better for boys carrying more enzyme related plasticity alleles than boys carrying less of such alleles (Chhangur
et al., in press) might therefore indicate that specifically differences in the degradation of dopamine, and in turn, the availability of dopamine in the presynaptic neuron and synaptic cleft is responsible for the differences in intervention effectiveness of IY. However, because both polymorphisms of interest have seemingly opposing effects on dopamine availability, it remains unclear whether the previously found G×I is due to a cumulative effect of the two polymorphisms or to one versus the other.

Another important question is how the COMT and/or MAOA polymorphisms influence the effectiveness of IY. Dopamine is related to reward sensitivity, and extant literature suggests that dopamine release during anticipation of reward causes a pleasant arousal (Pessiglione et al., 2006; Schultz, 2010). High dopamine availability may therefore result in increased arousal in response to rewards (Dreher et al., 2009; Lancaster et al., 2012), increased salience of rewarding experiences (Buckholtz et al., 2010), and, indirectly, increased effects of reward-based learning (Comings & Blum, 2000). As a result, children carrying polymorphisms upregulating dopamine might be specifically susceptible to the effects of reward oriented parenting strategies -such as praise and token economies- when compared to children with low dopamine availability (Matthys et al., 2012).

An important purpose of behavioral parenting training such as IY is to teach parents positive and reward-focused strategies to increase compliant and socially desirable behavior in their children. Parents are instructed on how to positively reinforce desired behavior in their children through using praise, sticker charts for charting positive child behavior and tangible rewards (Webster-Stratton, 2008). Specifically praise -verbalizations meant to socially reward children- is used in most, if not all, BPT programs (Maughan, Christiansen, Jenson, Olympia, & Clark, 2005; McCart et al., 2006), and has been shown to have a positive effect on child compliance (e.g., Eisenstadt, Eyberg, McNeil, Newcomb, & Funderburk, 2010; Marchant, Young, & West, 2004). IY might thus indirectly reduce child externalizing problems through an increase in the use of positive reward oriented parenting and decrease in negative punishment oriented parenting: The intervention effect might be mediated by changes in this specific parenting behavior. Indeed, our previous effectiveness study showed that IY led to an increase in reported and observed positive parenting strategies and a reported (but not observed) decrease in negative parenting strategies (Weeland & Chhangur et al, submitted for publication, Chapter 4). However, these strategies might not be equally effective for all children. Children with genotypes related to high dopamine availability might be specifically reward sensitive, and thus more susceptible to reward-based parenting strategies such as praise. In turn, this may cause these children to decrease more in oppositional and defiant behavior and to increase more in prosocial, positive behavior then children who are less genetically susceptible.
Present study
The goals of this study were: (1) to further explore a previously reported Gene×Intervention (G×I) interaction between the evidence-based Incredible Years program (IY) (see Chapter 4 for reports on the effectiveness of IY) and a polygenic dopaminergic index consisting and enzyme- related polymorphisms (COMT, MAOA) in boys, and (2) to test whether this interaction could be explained by genetic differences in reward sensitivity. In a three wave (pre-test, 6 month post-test and 10 month follow-up) randomized controlled trial (RCT) we therefore examined whether the effect of the behavioral parent program IY on change in parent-reported child externalizing behavior might be mediated by observed change in parents’ use of praise (a reward based parenting strategy). We used different informants for the mediator (observed praise) and outcome (reported child externalizing problems), which is important because it prevents bias and method dependence between these variables. Furthermore, we tested whether the increased use of praise by parents might be especially effective for children characterized by the low-activity allele of the MAOA gene and/or Val-alleles of the COMT gene. We expected that these polymorphisms would alter the (praise-mediated) effects of IY because they predispose children for increased reward sensitivity.

Methods and materials
Participants
Participants of this study were 387 parent-child dyads who participate in the Observational Randomized controlled trial of Childhood Differential Susceptibility (ORCHIDS study) (see Chhangur & Weeland, et al., 2012, Chapter 3). 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). For more information on the sample see Weeland & Chhangur and colleagues (submitted for publication, Chapter 4).

Procedure
Participants were recruited through two Dutch regional health care organizations in two separate cohorts. All families in the targeted municipalities with children between 4 and 8 years of age were screened using the Eyberg Child Behavior Inventory (Eyberg, & Pincus, 1999). When children scored at or above the 75th percentile of their respective cohort they were invited to participate in the trial. The RCT consisted of three measurement waves (i.e., pre-test, post-test after 6 months and follow-up after 10 months). Parent and child behavior was assessed using both questionnaires and observations at all three waves. At the first wave a buccal swab was collected from children. Approval was received from the Institutional Review Broad in The Netherlands (METC UMC Utrecht, protocol number 11-320/K). For
more information on the recruitment procedure see Weeland & Chhangur and colleagues (submitted for publication, Chapter 4).

**Intervention: The Incredible Years BASIC program.**

After the pre-test, parents were randomly assigned into two groups (i.e., control or intervention). Parents in the intervention condition received The Incredible Years program (IY, Webster-Stratton, 2008) between pre-test and post-test. Parents in the control condition did not receive any intervention through the trial, but were free to seek additional help (i.e., “care-as-usual”). The IY program is developed to prevent and intervene in the development of child externalizing problems by reducing harsh and unresponsive parenting and increasing positive and warm parenting. The program has been studied intensively and was found effective in reducing child externalizing problems across settings and target populations (for a meta-analysis see Menting et al., 2013). IY consists of 15 sessions (i.e., 14 weekly 2-hour sessions and a “booster” session) and promotes the use of positive parenting strategies such as play, praise and incentives before discussing effective limit setting, ignoring unwanted behavior, and finally, time out strategies (Webster-Stratton, 2008). Participants of the ORCHIDS study intervention group attended, on average, 8.6 sessions; 44 parents in this group did not attend any sessions. These parents did not differ from parents that did attend sessions (see Weeland & Chhangur et al., submitted for publication, Chapter 4).

**Measures**

**Child Externalizing Problems**

The Eyberg Child Behavior Inventory (ECBI) assesses the occurrence of conduct problems in children aged 2 to 16 years (Eyberg, & Pincus, 1999). The ECBI intensity scale was used consisting of 36 items (e.g., ‘Acts defiant when told to do something’) rated by parents on frequency using a 7-point scale (1= never to 7= always). Reliability of the scale was good for all three waves (α >.84).

**Praise**

Parents’ use of praise was assessed through observation using the Dyadic Parent–child Interaction Coding System (DPICS). The DPICS is a home observational measure for parent–child interactions, which assesses the quality of the social interaction (Robinson & Eyberg, 1981; Webster-Stratton, 1989). Parent and child were observed for 20 minutes while playing with a fixed set of toys at pre-test, post-test and follow-up. The observation procedure consisted of 4 five-minute periods: free play (i.e., to get used to being videotaped); child directed play (i.e., child picked a toy and directed the session); parent directed play (i.e., parent picked a toy and directed the session); and clean up (i.e., parent had to make
the child clean up). For the last three periods, the number of times parents used labeled or unlabeled praise was scored (which ranged between 0 and 41 times).

The observations were coded by trained research assistants who were not involved in the study and who were blind to families’ condition as well as the measurement wave. To prevent observer drift monthly calibration meetings were held. In addition, two coders independently coded at random 20% of the observations to assess observer agreement. Coders were unaware of which observations were used to assess this. Intraclass correlations (ICCs, using SPSS 22.0) showed intrarater reliability was excellent at all measurement waves; ICCs were > .85 for all positive parenting strategies.

**Genotyping**

Buccal swabs were collected in lysis buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/ml proteinase K and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). Within the ORCHIDS study six genes were analysed (i.e., MAOA, COMT, DRD2, DRD4, DAT1, and 5-HTTLPR) (for more information on the procedure see Chhangur et al., in press). The focus of the current paper is specifically on the MAOA and COMT polymorphisms, since our previous studies found these polymorphisms to moderate the intervention effects (see Chhangur et al., in press; submitted for publication). Results for the 5-HTTLPR polymorphism are reported elsewhere (Weeland et al., submitted for publication, Chapter 5).

**MAOA.** The region of interest from the MAO-A gene was amplified by PCR using the following primers: an FAM-labelled MR primer (5’-GGATAACAATTTCACACAGG-3’), forward primer (5’-ggataacaatttcacagACAGCTGGACCCTGGAGAAG-3’) and a reverse primer (5’-GGACCTGGCGAGTGTGTC-3’). Typical PCR reactions contained between 10 and 100 ng genomic DNA template, 1 pmol of forward primer, and 10 pmol of labelled MR and reverse primers. PCR was carried out in the presence of 5% DMSO with 1.25U of LongAmp Taq DNA Polymerase (NEB) in a total volume of 30 μl using the following cycling conditions: initial denaturation step of 5 min at 94°C, followed by 38 cycles of 30 sec 94°C, 30 sec 55°C, 30 sec 72°C and a final extension step of 4 min 72°C. One microliter of PCR product was mixed with LIZ-500 size standard and formamide and run on a ABI 3730 genetic analyser set up for genotyping with 50 cm capillaries. Results were analysed using GeneMarker software (Softgenetics). The MAOA genotype distribution was 64.5% high/high; 31.9% high/low and 3.7% low/low genotype (n = 17 no genotyping), and did not differ between participants of the control and the intervention condition ($\chi^2 = 4.64; df = 2; p = .10$). Genotypes for boys were $n = 80$ low and $n = 123$ high. Since boys have only one X chromosome, Hardy-Weinberg equilibrium (HWE) calculation were done for girls only.
Analyses

To assess changes in child and parenting behavior over the three time points (pre-test, post-test and follow-up) latent growth curves were modeled (LGCM) using Mplus 7 (Muthén & Muthén, 1998-2015). LGCM was chosen because it estimates an individual growth curve (i.e., slope) for each child and parent, making it possible to examine variation in the development of the outcome variables. Full information maximum likelihood (FIML) was used to treat missing data. For this study we only used the data for boys (n = 214). Families in the intervention condition who did not attend any sessions (n = 22) were excluded from the analyses (i.e., “completers only” analysis). Model fit of the growth curves was assessed using the Root Mean Square Error of Approximation (RMSEA) (model fit satisfactory when < .08) and Tucker-Lewis Index (TLI) and Confirmatory Fit Index (CFI) (model fit satisfactory when > .90) (Hu & Bentler, 1999).

The analyses were performed in three steps. In step 1, we tested moderation of the intervention effect by children’s MAOA and COMT genotype. LGCM of child externalizing problems was modeled to assess whether participants in the intervention condition significantly differed from participants in the control condition in development of child and parenting behavior over time. Main effects of MAOA and COMT genotype, as well as condition × genotype interactions, were added to assess possible moderation effects of MAOA and COMT genotype. We performed two robustness checks for significant G×I effects: First, it has been shown that heteroscedasticity can masquerade G×E effects (Salvatore & Dick, 2015). Therefore, we repeated the analysis using nonlinear transformations of externalizing problems (i.e., logarithmic transformations). Second, it has been argued that G×E studies have not appropriately controlled for covariates and covariate interactions, particularly in mixed-ethnicity samples (Dick et al., 2015; Keller, 2014). Due to the experimental nature of our study, we can rule out effects of many possible covariates such as socio-economic status (for a report on non-genetic moderators of the intervention effect Weeland & Chhangur et al., submitted for publication, Chapter 4). However, due to possible population stratification it...
might still be important to take into account child ethnicity (Enoch et al., 2006). Therefore, we repeated the analysis adding child ethnicity (birth country of parents: The Netherlands or other) and ethnicity × genotype covariates (conform Keller, 2014).

In step 2, we tested whether the intervention effect on child externalizing problems is explained by changes in parents’ use of praise (Preacher, Rucker, & Hayes, 2007). We used parallel process LGCM to test mediation, testing an indirect effect from intervention condition to the slope of child externalizing problems via the slope of praise (see Cheong, Mackinnon, & Khoo, 2003; Kauer et al., 2012). For the sake of completeness all direct pathways (a) condition to slope child externalizing problems; (b) condition to slope parental praise; and (c) slope parental praise to slope child externalizing problems are reported alongside the indirect effects. However, these direct effects are no statistical prerequisite for the existence of indirect effects (Preacher & Hayes, 2008).

In step 3, we tested whether the intervention effect on child externalizing problems is explained by changes in the parental use of praise, specifically for specific genotypes. We reran the model of step 2 using a multi-group approach. To test whether the models were significantly different across groups, we used the chi-square difference test ($\Delta\chi^2$) to compare the models in which all paths were constrained to be equal across groups with the free models in which all paths were estimated freely. Evidence for moderated mediation was found if the constrained model fit significantly worse than the unconstrained model. For the models that indicated significant group differences, we compared the specific path coefficients between the groups, taking into account sample size and standard errors for each group (Cohen, Cohen, West, & Aiken, 2013; Soper, 2016).

Results

Preliminary Analyses.

Table 5.1 shows descriptive statistics for child externalizing problems and parental praise. Bivariate associations showed that at pre-test none of the constructs were significantly correlated. Exploration of the data showed that there were no significant outliers. However, praise was positively skewed. Therefore, maximum likelihood robust (MLR) fit indices will be reported. Furthermore, bootstrapped (5000 bootstrap samples) confidence intervals were used to evaluate significance of indirect effects.

Step 1. Moderation.

LGCM of child externalizing problems showed sufficient model fit: $\chi^2(N = 192, 7) = 14.12$, CFI = .97, TLI = .92, RMSEA = .075. None of the predictors (i.e., condition, MAOA, COMT and their interaction variables) were significantly related to the intercept of externalizing behavior problems, meaning there children in the control and experimental group and
characterized by different genotypes had comparable levels of externalizing behavior. Controlled for effects of the COMT polymorphism, only the condition × MAOA interaction had a significant effect on the slope of externalizing problems (Table 5.2). This interaction effect indicates that the intervention had a significant effect on decreasing child externalizing problems for children with a low-activity variant of the MAOA genotype ($B = -0.35$, s.e. = .08, $p < .001$, partial $\eta^2 = .13$), but not for children carrying the MAOA high-activity allele ($B = -0.05$, s.e. = .06, $p = .36$, partial $\eta^2 = .00$; coefficient difference between genotypes: $t = 3.0$, $df = 179$, $p < .01$) (see Figure 5.1 for illustration of the interaction).

![Figure 5.1 / Illustration of the development of child externalizing problems across three time-points (1 = pre-test; 2 = post-test; 3 = follow-up), for the two MAOA genotypes (low and high activity allele) in the two conditions (intervention and control).](image)

**Table 5.1 / Descriptive Statistics Child Externalizing Problems and Observed Parental Praise in Boys (n = 192).**

<table>
<thead>
<tr>
<th></th>
<th>Pre-test (T1)</th>
<th></th>
<th>Post-test (T2)</th>
<th></th>
<th>Follow-up (T3)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Intervention</td>
<td>Control</td>
<td>Intervention</td>
<td>Control</td>
<td>Intervention</td>
</tr>
<tr>
<td></td>
<td>$M$</td>
<td>$SD$</td>
<td>$M$</td>
<td>$SD$</td>
<td>$M$</td>
<td>$SD$</td>
</tr>
<tr>
<td>Child externalizing problems</td>
<td>3.77 .51</td>
<td>3.88 .48</td>
<td>3.62 .57</td>
<td>3.48 .51</td>
<td>3.51 .56</td>
<td>3.45 .57</td>
</tr>
<tr>
<td>Parental praise</td>
<td>5.07 4.54</td>
<td>6.66 5.41</td>
<td>4.67 3.69</td>
<td>11.85 9.69</td>
<td>4.16 3.16</td>
<td>9.85 7.58</td>
</tr>
</tbody>
</table>
Chapter 5

characterized by different genotypes had comparable levels of externalizing behavior. Controlled for effects of the COMT polymorphism, only the condition × MAOA interaction had a significant effect on the slope of externalizing problems (Table 5.2). This interaction effect indicates that the intervention had a significant effect on decreasing child externalizing problems for children with a low-activity variant of the MAOA genotype ($B = -0.35$, s.e. = .08, $p < .001$, partial $\eta^2 = .13$), but not for children carrying the MAOA high-activity allele ($B = -0.05$, s.e. = .06, $p = .36$, partial $\eta^2 = .00$; coefficient difference between genotypes: $t = 3.0$, $df = 179$, $p < .01$) (see Figure 5.1 for illustration of the interaction).

Figure 5.1. Illustration of the development of child externalizing problems across three time-points (1 = pre-test; 2 = post-test; 3 = follow-up), for the two MAOA genotypes (low and high activity allele) in the two conditions (intervention and control).

Table 5.1 / Descriptive Statistics Child Externalizing Problems and Observed Parental Praise in Boys (n = 192).

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B intercept</th>
<th>s.e.</th>
<th>$p$</th>
<th>B slope</th>
<th>s.e.</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>.27</td>
<td>.16</td>
<td>.09</td>
<td>-.10</td>
<td>.10</td>
<td>.35</td>
</tr>
<tr>
<td>MAOA</td>
<td>-.18</td>
<td>.10</td>
<td>.24</td>
<td>.10</td>
<td>.06</td>
<td>.08</td>
</tr>
<tr>
<td>COMT</td>
<td>.16</td>
<td>.11</td>
<td>.14</td>
<td>-.06</td>
<td>.06</td>
<td>.36</td>
</tr>
<tr>
<td>Condition × MAOA</td>
<td>.16</td>
<td>.17</td>
<td>.27</td>
<td>-.28</td>
<td>.10</td>
<td>.01</td>
</tr>
<tr>
<td>Condition × COMT</td>
<td>-.23</td>
<td>.15</td>
<td>.18</td>
<td>.05</td>
<td>.12</td>
<td>.68</td>
</tr>
</tbody>
</table>

The interaction between condition and MAOA remained significant using log transformed scores on child externalizing problems $\chi^2(N = 192, 7) = 11.91$, CFI = .98, TLI = .94, RMSEA = .062; $B = -0.35$, s.e. = .01, $p = .01$, and when adding child ethnicity × MAOA interaction covariates to the regression model $\chi^2(N = 192, 7) = 14.38$, CFI = .97, TLI = .92, RMSEA = .076; $B = -0.29$, s.e. = .10, $p < .01$. Since these findings indicate that the enzyme based G×I reported in our previous study (Chhangur et al., submitted for publication) is solely carried by the MAOA genotype, the COMT gene was not included in further analyses.

Step 2. Mediation

Parallel process LGCM of child externalizing problems and parental praise overall showed sufficient model fit, although a lower RMSEA value was observed: $\chi^2(N = 192, 15) = 39.49$, CFI = .94, TLI = .92, RMSEA = .09. Results show that the intervention was successful in increasing parents’ use of praise (partial $\eta^2 = .19$). However, the change in parental praise did not predict the change in child externalizing problems, and there was no indirect effect from the intervention on child externalizing problems via praise (see Table 5.3).

Step 3. Moderated Mediation

Chi$^2$ difference testing of ML model fit showed no significant difference ($\Delta \chi^2 = 6.49$, $\Delta df = 3$, $p = .09$) between the constrained and free multi-group model. This means that the model fit did not improve when estimating the mediation pathways for the MAOA low- and high genotypes separately. This indicates that the effect of the intervention on parental praise and the effect of parental praise on child externalizing problems did not differ between carriers of the MAOA low- and high-activity allele.
Conclusion and Discussion

In a three wave (pre-test, post-test and follow-up) randomized controlled trial we further explored a previous reported Gene×Intervention (G×I) interaction between The Incredible Years program (IY) and a polygenic dopaminergic index consisting two enzyme-related polymorphisms (Chhangur et al., submitted for publication) and whether this interaction could be explained by genetic differences in reward sensitivity, specifically sensitivity to parents’ use of praise. We hypothesized that the MAOA and/or COMT polymorphism would alter the (praise-mediated) effects of IY because they predispose children for increased reward sensitivity.

Our findings show that the previously detected polygenic G×I was solely explained by the MAOA polymorphism. The intervention effects on child externalizing problems were stronger for boys carrying the MAOA low-activity allele, compared to boys carrying the high-activity allele for whom there was no significant effect of the intervention. This G×I remained significant when properly controlling for possible effects of the COMT polymorphism, child ethnicity, and heteroscedasticity. The finding that the effect of the environment is stronger for boys carrying the MAOA low-activity allele, than boys without, is seemingly in line with many previous studies on the MAOA (for a meta-analysis see Byrd & Manuck, 2014). On the one hand, our findings might indicate that these children are more sensitive to environmental enrichment, and therefore show vantage sensitivity (Pluess & Belsky, 2013). On the other hand-combined with previous findings that compared to boys carrying MAOA the high-activity allele boys carrying the low-activity allele are more vulnerable when maltreated and in turn develop more conduct problems (e.g., Byrd & Manuck, 2014; Caspi et al., 2002)-our results might also be interpreted in line with differential susceptibility thinking: Boys carrying the low-activity allele seem not only 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 due to intervention).

We found no moderation of the intervention effectiveness by COMT genotype. This links to previous literature, in that findings on the COMT polymorphism in relation to externalizing behavior have been mixed (Weeland, Overbeek et al., 2015; Chapter 2). These
mixed findings might be explained by a cognitive/emotional trade-off (i.e., the warrior-worrier hypothesis, Goldman et al., 2005), in which the Val-allele is associated with an advantage in emotional processing and the Met-allele in cognitive processing (see Mier et al. 2009). The Met-allele (i.e., the worrier) is associated with an advantage for prefrontal cortex—and related cognitive—functioning. However, at the same time this allele might contribute to emotional dysregulation, an irritable mood and reactive aggression or mood disorders (Drabant et al., 2006; Thompson et al., 2012). It might therefore be that the COMT polymorphism is related to cognitive processes underlying differential effects of the environment, instead of processes related to socialization as we studied in our present analyses.

The question that remains: How can we explain the found G×I with the MAOA? Theoretically, our finding 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 as particularly strong behavioral reinforcers. Therefore, these children might specifically benefit from IY because they benefit substantially from an increase in such parenting behavior. Praise-verbalizations meant to socially reward children—is one of the best-represented strategies in BPT to enhance reward oriented parenting behavior (Maughan et al., 2005; McCart et al., 2006). Our results show that IY indeed caused a large increase in parents’ use of praise (partial $\eta^2 = .19$). However, in contrast with this explanation, the increase in parental praise 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.

One explanation might be that reward sensitivity is not a mechanism underlying the G×I between IY and the MAOA polymorphism. Besides degrading dopamine, monoamine oxidase A is also involved in degrading other neurotransmitters such as serotonin and norepinephrine. It has even been suggested that the effects of the MAOA on availability of these neurotransmitters are larger than the effects on dopamine (for an overview see Buckholtz & Meyer-Lindenberg, 2008). Therefore, other emotional-motivational traits, such as differences in the processing and regulation of emotions, are possibly responsible for differences in how (strongly) children react to changes in parenting behavior (see for an overview Moore & Depue, 2016). For instance, due to increased activation of the amygdala and dorsal anterior cingulate cortex, and increased connectivity between the two, when exposed to anger related stimuli, boys carrying the MAOA low-activity allele might experience increased difficulties in anger control, compared to boys carrying the high-activity allele (Denson, Dobson-Stone, Ronay, Von Hippel, & Schira, 2014). When dealing with for example an angry parent, these boys might find it difficult to shift their attention away from the sources of irritation, control their negative emotions and in turn control.
their negative behavioral responses. These boys might therefore be particularly sensitive to negative and harsh parenting strategies, but at the same time particularly sensitive to an intervention induced decrease in parental self-control, anger and harshness.

Alternatively, our findings might indicate that the parenting strategy ‘praise’ alone does not capture reward oriented parenting strategies. In our study, parents’ use of praise was not related to child behavior. Research findings on the effect of praise on child behavior have been mixed (for a review see Owen, Slep, & Heyman, 2012). It might therefore be that praise alone is not a predictor of child behavior, and therefore not a mechanism of change in IY. Different and multiple parenting strategies are part of the category “reward oriented” parenting such as nonverbal positive reinforcement (e.g., hugging), tangible rewards, and using token economy (e.g., tangible rewards). These parenting strategies were not included in our parent-child observation, leaving us unable to test their effects. However, mediation analyses using a composite score for positive parenting strategies, reported in a different paper, also failed to explain intervention effectiveness of IY on child externalizing problems (see Chapter 6).

Irrespective of the explanation for our lack of support for the putative mechanism of the found G×I, our study-results add to the view that the mechanisms underlying interactions between our environment and biology might be very specific and complex (Moore & Depue, 2016; Reiss et al., 2013). Furthermore, due to ongoing interactions between genetic dispositions and the environment, these mechanisms might become increasingly complex over developmental time. Testing such mechanisms at an early developmental period might prove more successful. Nevertheless, gaining insight into the underlying biopsychosocial mechanisms of G×I is of great importance. In order to be able to grant any empirical or clinical implications to findings on G×I we are in need of more knowledge on the neurobehavioral associates of the genetic markers we use.

A few important limitations of our study should be acknowledged. First, although our sample size is at least comparable to other G×I studies (a recent meta-analysis on G×I showed an average N of 148), it is still a relatively modest sample size to test complex models such as moderation and mediated moderation. A larger sample size, and therefore more adequately powered models, might have yielded different results. Second, our present randomized controlled trial is based on a relatively specific subsample of native Dutch families willing to be enrolled, randomized and to attend 14 weekly intervention sessions. Our results may therefore not be generalizable to the general population or to other countries. Third, although our current study included a four-month follow-up assessment, no generalization of IY intervention effects across longer time intervals could be observed. Specifically, inspection of Figure 5.1 shows that eyeballing the results there seems to be a further differentiation of intervention effects on child externalizing problems behavior between carriers of the MAOA high- and low-activity allele. Because we only followed children up to four months
post intervention, we do not know whether these differential effects will continue across a longer time frame. Fourth, although the use of an observed, instead of a parent reported, mediator can be considered a major strength of this study, at the same time it limited our mediator in validity, range, and reliability. We only have observational data on parenting behavior during a brief play situation, which might decrease validity of this measure to capture day-to-day variability in parenting behavior (see Weeland & Chhangur, submitted for publication, Chapter 4).

Our findings add to the literature in multiple ways. First, we used a three wave randomized controlled trial to test G×E. This design enables us to rule out alternative explanations for interaction effects, such as correlations between environment and child characteristics. It thus provides robust, causal evidence for such interactions. Second, we used different informants for the mediator (i.e., observed praise) and outcome (i.e., parent-reported child externalizing problems). This is important since mediation is often assessed using parent reports of both parenting behavior and child externalizing problems, possibly causing inflated observed associations due to shared informant variance. Third, using an evidence based intervention, our study is part of a growing body of literature on G×I findings, showing that individual characteristics such as one’s genetic make-up might be important predictors of the effectiveness of a particular intervention. Failure to take into account these characteristics might lead to over- or underestimation of intervention effects.

In sum, our findings suggest that, although a primitive approach, some candidate genes might be used as markers of individual differences in susceptibility to interventions. Single candidate gene studies have been criticized for their naïve approach of genetics in human development (Chabris et al., 2013; Szyf & Bick, 2013). Ironically, our attempt to combine all available genetic information on dopaminergic functioning eventually brought us back (full circle) to a single candidate gene. Our study shows that the previously found polygenic effects on intervention effectiveness (Chhangur et al., in press) are due to the effect of a single polymorphism, namely the MAOA. However, this does not necessarily imply that the mechanisms underlying such G×I interaction are not more complex. cG×I interactions might just be simplistic renderings of more intricate processes including multiple (neuro) biological, psychological, and social factors. Moreover, from a clinical perspective, it remains unclear whether and how information on genetic moderation should be used in practice. Given replication issues in this field, any practical application of a genetic marker would at the very least require multiple replications. Furthermore, using genetic information to identify children who might be more or less susceptible for a certain intervention is costly, invasive, and yields ethical concerns (Ross, Saal, David, Anderson, & American Academy of Pediatrics, 2013). Genetic screening may have negative side effects, such as false deterministic claims about child externalizing problems.