My dopamine has been busy: Research on gene by environment interactions in child externalizing behavior

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MY DOPAMINE HAS BEEN BUSY

RABIA CHHANGUR

RESEARCH ON GENE BY ENVIRONMENT INTERACTIONS IN CHILD EXTERNALIZING BEHAVIOR
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Research on Gene by Environment Interactions in Child Externalizing Behavior

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit van Amsterdam op gezag van de Rector Magnificus prof. dr. ir. K.J.J. Maex ten overstaan van een door het College van Promoties ingestelde commissie, in het openbaar te verdedigen in de Aula der Universiteit op woensdag 5 oktober 2016, te 13.00 uur door Rita Rabiagatoen Chhangur geboren te Paramaribo, Suriname
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               Prof. dr. S. M. Bögels, Universiteit van Amsterdam
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GENERAL INTRODUCTION
CHAPTER 1
GENERAL INTRODUCTION

There is consensus that parents play a pivotal role in how their children develop and function (Karreman, Van Tuijl, Van Aken, & Dekovic, 2006; Rothbaum & Weisz, 1994). Many of the behavioral skills young children and adolescents learn are highly dependent on the quality of parenting they receive and evoke (Miner & Clarke-Stewart, 2008; Stormshak, Bierman, McMahon, & Lengua, 2000). In fact, parenting is considered to be one of the strongest potentially modifiable risk factors that contributes to the development of child externalizing behavior (McCart, Priester, Davies, & Azen, 2006). Yet more recent research suggests that not all children are equally sensitive to their environment and, thus, to the parenting they receive. A growing body of recent evidence demonstrates that genes might have something to do with this phenomenon (Belsky & Pluess, 2009, 2015). Indeed, some studies suggest that children carrying a genetic “polymorphism” seem to be more sensitive to quality of parenting than children without such a polymorphism (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015). The word polymorphism comes from the Greek roots “poly” (many) and “morphe” (form). It implies that some individuals have a variation in a single gene (i.e., DNA sequence) that is relatively common in the population, being prevalent in at least 1%. Such a variation in a gene might alter neurobiological processes in the brain that possibly make children more sensitive to their environment (Matthys, Vanderschuren, & Schutter, 2013).

It is specifically this interaction between a gene and the environment (i.e., G × E) that is thought to shape externalizing behavior. However, G × E findings have raised criticism and serious concerns regarding mixed findings and replications, making it difficult to draw conclusions (Dick et al., 2015; Duncan & Keller, 2011; Jaffee, Price, & Reyes, 2013; Weeland, Overbeek, de Castro, & Matthys, 2015). One important challenge is to fill in the details in neurobiological processes that link genes and environment to child externalizing behavior (Moore & Depue, 2009; Salvatore & Dick, 2015). By addressing specific neurobiological components related to environmental sensitivity, studies would contribute to a better understanding of G × E interactions (Chorpita & Daleiden, 2009, Tolan, Dodge, & Rutter, 2013). Another relevant challenge pertains to G × E confounders (Keller, 2014). Many G × E studies use correlational designs (Riley, 2008) that do not permit causal inferences and are unable to rule out alternative interpretations in terms of gene-environment correlations (i.e., rGE). Longitudinal and experimental studies can overcome these concerns by design (Bakermans-Kranenburg & Van IJzendoorn, 2015). The aim of this thesis is to clarify G × E interactions in child externalizing behavior based on multiple genes influencing the dopamine system.

First, we conducted a longitudinal study to predict G × E over time and to statistically account for passive rGE. Second, an intervention study was carried out to experimentally manipulate the environment, thereby increasing statistical power, ruling out any rGE confounding, and reducing environmental measurement error.

Externalizing behavior in children

Mild levels of externalizing behavior (e.g., aggression, oppositional behavior,
disobedience) are considered to be typical behavior in young children. However, some children show high levels of externalizing behavior that, when left untreated, might worsen with age and might develop into persistent patterns of serious anti-social behavior (Campbell, Shaw, & Gilliom, 2000). In fact, stable high or increasing levels of externalizing behavior in early childhood might be a sign of incipient severe externalizing behaviors in adolescence, including delinquency (Fergusson, Boden, & Horwood, 2014; Miettunen et al., 2014; Moffitt, 2003). Such forms of externalizing behavior carry substantial social and economic costs to individuals and society (Raasackers, Posthumus, Van Hout, Van Engeland, & Matthys, 2011; Scott, Knapp, Henderson, & Maughan, 2001). Moreover, families may experience adverse effects of the child’s externalizing behavior and might be hindered in their daily functioning, resulting in marital discord, parental stress, and productivity losses (e.g., Mackler et al., 2015). Not surprisingly, then, the developmental legacy of externalizing behavior underscores the need to learn more about the causes of externalizing behavior in childhood.

Parenting behavior and externalizing behavior

Extensive evidence supports the notion that environmental characteristics, such as parenting behavior, are related to child externalizing behavior (Kaneman et al., 2006, Rothbaum & Weisz, 1994). Negative parenting behavior, like harsh and inconsistent discipline, limited use of praise, and lack of attention to appropriate behaviors are associated with higher levels of externalizing behavior in young children (e.g., Patterson, DeBaryshe, & Ramsey, 1989; Pettit & Bates, 1989; Shaw, Keenan, & Vondra, 1994) and adolescents (Hooe et al., 2009; Steinberg, Lamborn, Darling, Mounts, & Dornbusch, 1994). Although mild levels of externalizing behavior can be considered typical in early childhood, parents’ ineffective reactions might inadvertently result in more conflicts, leading to a fertile ground for children to become generally oppositional. As such, these children may learn to ignore demands that are unrewarding or unpleasant, thereby triggering a coercive exchange with their parents (e.g., Patterson, 1982; Scaramella & Leve, 2004). Positive parenting behavior, in contrast, is associated with decreases in externalizing behavior (e.g., Stormshak et al., 2000). Indeed, parents with a greater capacity of positive parenting qualities, including the use of tangible rewards, praise and other positive reinforcements, appear to be able to respond to externalizing behavior in a more predictable and consistent manner, thereby ameliorating early emerging problems and allowing their children to return to adaptive functioning (e.g., Gardner, Ward, Burton, & Wilson, 2003; Sandler, Schoenfelder, Wolchik, & Mackinnon, 2011). Thus, negative parenting may serve as an environmental risk factor in the development of externalizing behavior, but positive parenting may also serve as a protective factor.

**Gene × Environment interactions**

Behavioral genetic studies suggest a genetic contribution to the development of externalizing behavior in children. Such research has traditionally used twin and adoption studies, showing that the heritability of externalizing behavior ranges between 40% and 60% (Hicks, Foster, Iacono, & McCue, 2013; Rhee & Waldman, 2002). However, the emergence and persistence of externalizing behavior appears to be explained best by the interaction of genes with environment (e.g., Rutter, 2012). That is, children’s likelihood to develop externalizing behavior as a consequence of negative parenting behavior, depends in part on their genetic make-up. Specifically, genes related to dopaminergic brain functions would seem to play a role because of its link with reward-based learning and reward sensitivity (Bakermans-Kranenburg & Van IJzendoorn, 2011). Several dopaminergic polymorphisms—like the DRD2 A1, DRD4 7-repeat, DAT1 10-repeat, MAOA low-activity, and the COMT val—may contribute to differential sensitivity in responsiveness to parenting behavior and thereby to child externalizing behavior (e.g., Boardman et al., 2014; Ficks & Waldman, 2014; Wagner et al., 2010; Windhorst et al., 2015; Yang et al., 2007). These findings, however, have not always been straightforward (Dick et al., 2015; Jaffe et al., 2013) and have proven to be difficult to replicate (Duncan & Keller, 2011). This difficulty with replicability may in part be caused by the fact that previous studies are typically cross-sectionally designed and/or used a limited single candidate gene approach. In this regard, the use of (1) longitudinal and experimental designs, (2) the creation of polygenic indices based on multiple genes influencing a specific biological system (i.e., systems approach), and (3) linking the biological system to environment and child behavior would help to draw stronger conclusions about how G × E interactions relate to externalizing behavior.

**Longitudinal and experimental designs**

Longitudinal G × E studies use repeated-measurements that allow the investigation of how externalizing behavior unfolds over time and whether parenting behavior indeed predicts change in such behavior. However, although longitudinal G × E studies are invaluable sources for information they cannot rule out alternative explanations for detected G × E. One explanation lies in passive G × E. Passive G × E refers to the notion that the child’s exposure to the environment (parenting quality) is not random but rather influenced by his or her parents who carry the same genetic polymorphism (Dick, 2011; Horwitz & Neiderhiser, 2015). Longitudinal G × E studies could account for passive G × E in order to investigate whether interactions really constitute G × E evidence or are confounded by rGE. In addition, experimental G × E studies have advantages relative to longitudinal G × E studies due to their randomized experimental character: (1) this eliminates any concerns about rGE because in a randomized design, participants’ genes cannot be correlated with the (manipulated) environment, (2) experimental studies work with standardized environmental conditions that reduce environmental measurement error, and (3) experimental studies provide considerably more statistical power because the environmental variance is increased and—especially in the case of intervention studies—often use “at risk” samples (Bakermans-Kranenburg & Van IJzendoorn, 2015).
A dopamine-related systems approach

Until now, single candidate G × E studies have found only modest G × E effects (e.g., Dick et al., 2015). Moreover, results of these studies have generally not been consistently replicated in follow-up studies (Duncan & Keller, 2011; Jaffee et al., 2013). However, it may be that the functional contribution of genes are polygenic in nature – with each polymorphism of each gene making only a small contribution (Chen et al., 2011). The cumulative consideration of multiple genes, via polygenic indices, may collectively account for significant polygenic effects (Nikolaou, Ferrell, Manuck, & Hanin, 2011). Thus, the creation of polygenic indices that use multiple genes influencing a specific biological process may be a more accurate measure of children’s latent differential sensitivity to parenting behavior. In addition, different genes may also impact different aspects of the dopamine system by either affecting the amount of dopamine released (i.e., receptors), recaptured (i.e., transporters) or degraded (i.e., enzymes) (Chen et al., 2011).

Such further functional distinctions have not been considered much in G × E research but could potentially provide new information about how specific dopaminergic processes depend on genetic variability and how this, in turn, determines children’s behavioral responses to positive and negative parenting behavior.

Linking the dopamine system to parenting and externalizing behavior

Dopamine is a neurotransmitter that modulates, via dopamine signaling, reward processing (Schultz, 2002). Therefore, dopamine appears to be critical in reward sensitivity and reward-based learning (e.g., Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006; Schultz, 2010). In simplistic terms, dopaminergic neurons in the ventral tegmental area/substantial nigra provide information about whether the environmental stimulus is rewarding (reward salience) and, if so, the nucleus accumbens mediates the rewarding effect and provides information about whether or not specific behavior should be repeated (reward-based learning) (Dichter, Damiano, & Allen, 2012; Wise, 1996). The DRD2 A1, DRD4 7-repeat, DAT1 10-repeat, and COMT val polymorphism (but not MAOA low-activity) have been related to less dopamine signaling and impaired reward processing, resulting in reduced reward salience and reward-based-learning (Comings & Blum, 2000; Schultz, 2002). Indeed, decreased dopaminergic functioning has been observed in young children and adolescents showing severe externalizing behaviors (Matthys et al., 2013). As a consequence, children with decreased dopaminergic functioning may lack motivation to obtain ordinary and/or delayed rewards, making it difficult to learn adequate social behavior. Moreover, these children may actively seek stronger rewards in their environment to overcome a condition of stimulation seeking (Matthys, Vanderschuren, Schutter, & Lochman, 2012).

Diathesis-stress, differential susceptibility, and vantage sensitivity

Traditionally, differential sensitivity in responsiveness to parenting has been cast in diathesis-stress terms (e.g., Zuckerman, 1999), stipulating that some “vulnerable” children are more likely than others to develop problematically in response to adverse environmental context only. More recently though, a differential susceptibility theory has been put forward (Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2007; Belsky & Pluess, 2009, 2015; Boyce & Ellis, 2005; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2011), which postulates that those most vulnerable to adversity may also benefit the most from environmental enrichment. A related but different perspective is the vantage sensitivity framework (Pluess & Belsky, 2013; Pluess, 2015), which stipulates that some children as a function of their very personal characteristics, may exclusively be responsive to environmental enrichment. These frameworks need to be considered in G × E research to better understand how G × E interactions work in response to both positive and negative parenting behavior.

Genetic moderation of intervention efficacy

There is much interest in whether the experience of environmental enrichment that brings about decreases in child externalizing behavior is due to the same genetically induced qualities related to negative changes brought about by adverse environmental influences (i.e., differential susceptibility). This hypothesis can be investigated in intervention studies that use parents as primary agents for positive change in parenting behavior (Bakermans-Kranenburg & Van IJzendoorn, 2015). Indeed, so-called G × I (gene-by-intervention) research suggests that some children who may be more sensitive to adverse environmental influences, based on the allelic variants, may also be more sensitive to intervention-induced enrichment (Belsky & Pluess, 2009, 2013). However, more research on genetic moderation is required to investigate heterogeneity in intervention response, but also to investigate specific neurobiological processes allowing for genetic variability in response to induced positive parenting changes. Since the Incredible Years (IY; Webster-Stratton, 2001) parent intervention is one of the most effective behavioral parent training programs to prevent and/or ameliorate externalizing behavior in young children (Menting, Orobio de Castro, & Matthys, 2013), IY is the specific focus of this thesis regarding the investigation of genetic moderation of intervention efficacy.

Aims and outline of this thesis

The aim of this thesis is to examine genetic variability in susceptibility to negative and positive parenting behavior in a sample of children at risk for maintaining or developing externalizing behavior. First, a longitudinal study was carried out on an
existing dataset involving adolescents. Second, an experimental intervention study was carried out to intervene in early emerging externalizing behavior in young children. Rather than focusing only on single dopaminergic candidate genes as a potential moderator of intervention effects, we adopted a systems approach by creating a dopaminergic polygenic index.

In chapter 2, we report on a longitudinal study in which we investigated the moderating role of the DRD2 and DRD4 in the longitudinal association between parental psychological control and parental support on the one hand and the development of delinquent behavior on the other hand, accounting for passive $KSE$. Indeed, high psychological control and low parental support have been studied as important predictors in the development of delinquency (Hoeye et al., 2009). We predicted that for DRD4 7-repeat carriers high perceived psychological control and low support would be more strongly related to the presence and development of delinquent behavior, when compared to their peers without such a polymorphism. Because of inconsistent effects in the DRD2 literature, in particular on delinquent behavior (e.g., Guo, Roetger, & Shih, 2007; Vasilyev, 2011), we explored whether either the DRD2 TaqI A1 or A2 allele would be associated with higher risk for the presence and development of delinquent behavior.

Left untreated, early externalizing behavior may worsen with age and tend to persist over time (Vaughn, Salas-wright, Delisi, & Maynard, 2013). This underscores the need for early intervention to ameliorate such early emerging problems. In chapter 3, we describe the study protocol for project ORCHIDS (Observational Randomized Controlled Trial on Childhood Differential Susceptibility), which is designed to examine heterogeneity in IY intervention effectiveness. In this study protocol we delineate the hypotheses about genetic moderation of intervention effects. The inclusion of genes in experimental intervention-based studies is fraught with difficulties and raises ethical questions. In chapter 4 we make some of these ethical questions explicit by discussing whether it is ethically responsible to withhold an effective treatment; to what extent or under which circumstances genetic data should be disclosed; whether researchers should be allowed to collect genes of both children and parents; and what costs and benefits of personalized interventions are based on (genetic) screening.

Heterogeneity in responses to intervention effects may be due to genetic variation (Belsky & Van Uzendoorn, 2015; Van Uzendoorn & Bakermans-Kranenburg, 2015). Since responsiveness to positive parenting change may depend on reward sensitivity/salience and reward-based learning (Matthys et al., 2015), it may very well be that some of the determinants of variation in intervention response depend on variance in dopaminergic genes. In chapter 5, we examine the effectiveness of the IY parent intervention program and potential moderators (i.e., initial severity of externalizing problem behavior, child gender, social economic status, family composition, and number of sessions parents attended), following the ORCHIDS design presented in chapter 3. The IY program was offered as an indicated preventive intervention in order to reduce externalizing behavior in young children. In this study, we predicted that parents assigned to the intervention group would improve more in positive parenting behavior than those assigned to the control group and that their children would show greatest decreases in externalizing behavior.

In chapter 6, we used the ORCHIDS study to investigate genetic moderation of intervention efficacy by creating a dopaminergic polygenic index. Genes were selected on a priori defined hypotheses (see chapter 5). We predicted that children scoring highest on a dopaminergic polygenic index (DRD2 A1, DRD4 7-repeat, DAT1 10-repeats, MAOA low-activity, and the COMT val allele) would show the greatest decrease in externalizing behavior in response to the IY intervention and that this would be especially so when parents evinced substantial rather than limited improvement in their positive parenting behavior. As all children were screened to have relatively high levels of externalizing behavior—presumably indicating an at risk group—we predicted that in the control group those children scoring high on the dopaminergic index would demonstrate greatest increases in externalizing behavior.

In chapter 7, we elaborate on the findings presented in chapter 6, by decomposing the dopaminergic polygenic index into receptors (DRD2, DRD4), transporters (DAT1), and enzymes (MAOA, COMT). This because these genes play a different role in dopamine signaling by respectively either modulating the amount of dopamine released (via neural signaling), recaptured, or degraded (Chen et al., 2011). As such functional distinctions have not been considered much in G x E research, we explored the proposition that one or more of the three dopaminergic subsets might be responsible for the polygenic moderation of IY efficacy.

In chapter 8, the results described in the previous chapters are summarized and discussed. In addition, the role of the dopaminergic system in a differential-susceptibility-related manner is discussed as well as recommendations for further G x E research.
DRD4 and DRD2 Genes, Parenting, and Adolescent Delinquency: Longitudinal Evidence for a Gene by Environment Interaction
**CHAPTER 2**

**DRD4 AND DRD2 GENES, PARENTING, AND ADOLESCENT DELINQUENCY: LONGITUDINAL EVIDENCE FOR A GENE BY ENVIRONMENT INTERACTION**

**KEYWORDS**
- Gene by environment interactions
- Delinquency
- DRD4
- DRD2
- Parenting

**ABSTRACT**

Gene by environment (G × E) research has been increasingly appreciated as it relates to the development of psychopathology. In particular, interactions between dopaminergic genotypes and maladaptive parenting have been prominently in the spotlight. In this study, we investigated whether high parental psychological control and low parental support would be differentially related to the development of delinquency in adolescents based on their genetic background (i.e., DRD4 and DRD2 genotypes). Data were derived from a 5-wave longitudinal survey among adolescents (N = 308; Mage = 13.4 at Time 1). After accounting for possible passive genetic effects (i.e., parents’ genotype, Parents’ Genotype × Adolescents’ Genotype, and Parents’ Genotype × Parenting, cf. Keller, 2014), latent growth modeling revealed a significant interaction of DRD2 × Parental Support, indicating that adolescents with the DRD2 A2A2 genotype were more vulnerable for low parental support, developing more delinquent behavior as a consequence. No significant interactions emerged for DRD4 with parental support and psychological control, nor for DRD2 with parental psychological control. The observed effect size of the identified DRD2 × Parental Support interaction was modest, emphasizing that replication is essential to confirm the present evidence.

Juvenile delinquency has high economic and social costs and impacts directly on social welfare, criminal justice, and health care systems (Scott et al., 2001). In addition, delinquency in adolescence is a known precursor of the development of serious violent crime and antisocial behavior in adulthood. Given these adverse consequences, it is pivotal to identify risk mechanisms underlying the development of delinquency in early adolescence. One of the strongest predictors of delinquency is harmful parenting behavior, such as high psychological control (Bean, Barber, & Crane, 2006) and low support (Barnes, Farrell, & Cairns, 1986). At the same time, specific genetic polymorphisms—located downstream of the DRD2 gene and in the DRD4 gene—have been associated with the development of aggression, conduct disorder, and other externalizing problem behaviors (Marino et al., 2004). However, we know relatively little about specific interactions between these biological and environmental risks. Also, we know relatively little about possible effects of parents’ genotypes that might provide an alternative account of observed gene by environment interactions G × E). That is, genetic relatedness between parent and their offspring (i.e., heritability of “risky genes”) may account for observed interactions between maladaptive parent- ing and adolescents’ genotype. In a 5-wave longitudinal study we (1) investigated G × E interactions involving variations of the DRD4 and DRD2 dopamine receptor genes with parental psychological control and support in the development of delinquency and (2) accounted for possible “passive genetic effects” (i.e., parents’ genotype, Parents’ Genotype × Adolescents’ Genotype, and Parents’ Genotype × Parenting), conform recent specifications by Keller (2014), that might influence the interactions.

Several behavioral genetic studies have demonstrated that monozygotic twins have a significantly higher concordance rate for delinquent behavior than dizygotic twins (e.g., Joseph, 2001), indicating that heritability plays a role in the development of delinquent behavior—but see Burt and Simons (2014) for a critique of methods and conclusions. In the search for genes that may be associated with the development of delinquency, previous studies have identified several dopamine-related candidate genes, among which the DRD4 and DRD2 dopamine receptor genes have received most attention (Elliott, 2000). Dopamine is an excitatory neurotransmitter related to motivation for obtaining rewards (Kelley, 2004), but also to the regulation of the anticipation of rewards (Blum et al., 1996). Studies suggest that delinquent behavior may be influenced by dopaminergic pathways in the brain (e.g., the ventral tegmental area, nucleus accumbens, and prefrontal cortex; Blum et al., 1996). Activation of these dopaminergic pathways may result in an intense feeling of pleasure or well-being and increased physiological arousal (Schultz, 2002). However, altered dopaminergic functioning in these pathways can affect motivation, reward processing, and consequently, the decision-making process may be aimed at increasing feelings of pleasure and physiological arousal (Matthys et al., 2013). Specifically, sensation seeking has been related to an altered functioning in dopaminergic pathways in the brain (Derringer et al., 2010, Harden, Quinn, & Tucker-Drob, 2012). Adolescents with a genetically induced disposition to seek exciting experiences, due to a blunted dopamine response to reward, may...
be at increased risk to develop delinquent behavior. The DRD4 and DRD2 genes are known to be involved in the regulation of these dopaminergic pathways and have been putative targets of studies on the etiology of externalizing problem behaviors, but how they are involved exactly is largely unknown (e.g., Elliot, 2000; Munafò, Yalcin, Willis-Owen, & Flint, 2008).

The DRD4 gene encodes the D4 subtype of the dopamine receptor. Main attention has been given to the 48-base-pair variable number of tandem repeats (VNTR) polymorphism in exon 3 of this gene, consisting of 2 to 11 repeats. Specifically, the 7-repeat allele is of interest, not only because of its association with dopaminergic functioning, but also because of its association with alcoholism (Laucht, Becker, Blomeyer, & Schmidt, 2007), attention-deficit/hyperactivity disorder (Li, Sham, Owen, & He, 2006), behavioral disinhibition (Congdon, Lesch, & Canli, 2008), novelty seeking (Ebbstein et al., 1996), and impulsivity (Eisenberg et al., 2007).

The DRD2 gene encodes the D2 subtype of the dopamine receptor which has a different structure than the DRD4 gene (e.g., receptor coding region contains six vs. three introns). The specific structure-activity requirements necessary to be selectively active at each receptor subtype are still unknown and need more investigation (see Missale, Nash, Robinson, Jaber, & Caron, 1998). Main attention has been given to a single-nucleotide polymorphism (SNP) with two variants at the TaqI A locus. This TaqI A locus is located 10 kb downstream from the DRD2 gene (Dubertret et al., 2004) and has been thought to be part of an adjacent protein kinase gene (i.e., ankyrin repeat and kinase domain containing 1, ANKK1, Neville, Johnstone, & Walton, 2004). Specifically, the TaqI A1 allele is of interest, not only because of its association with dopaminergic functioning (e.g., Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991; Pohjalainen et al., 1998), fewer D2 dopamine receptors (Berman & Noble, 1995), and decreased D2 binding (Noble, Gottschalk, Fallon, Ritchie, & Wu, 1997), but also because of its association with sensation seeking (Ratsma, Van der Stelt, Schoffelmeer, Westerveld, & Cunng, 2001), impulsive behavior (Eisenberg et al., 2007), and externalizing problem behaviors (Beaver et al., 2007). However, some other studies identified the TaqI A2 allele as marker for aggression (Vassilev, 2011), and inattentive and impulsive behavior (Rowe et al., 1999; Waldman, 2007). Also, a study based on the Add Health data found that DRD2 heterozygotes were most at risk for serious delinquent behavior in comparison with peers who carried either the A2/A2 or the A1/A1 genotype, respectively (Guo et al., 2007). These discrepant results are not uncommon in molecular genetic studies (see Lin, Vance, Pericak-Vance, & Martin, 2007; Marsman, Oldehinkel, Ormel, & Buitelaar, 2013; Waldman, 2007) and demonstrate that further research is needed to understand the relationship between the DRD2 TaqI variant and delinquency. This is especially needed given the fact that the ANKK1 and DRD2 genes are thought to be co-actors in a complex system of functionally related genes affecting the functioning of dopaminergic neurotransmitter pathways (Ponce et al., 2009). Genetic vulnerabilities may be expressed particularly when adolescents are exposed to maladaptive environmental factors, such as high parental psychological control and low support (Rutter, 2012). Decades of research informed by the diathesis-stress model of person-environment interactions showed that some individuals are more vulnerable to aversive effects than others because of individual characteristics, such as temperamental, physiological, or genetic factors (Zuckerman, 1999). Notably, Caspi and colleagues (2002) found that maltreated children with the low-activity allele of the MADI, more often developed conduct disorders than children with the high-activity allele of this gene. Ever since then, the notion that individuals may have an innate risk characteristic or diathesis that is expressed under aversive condition has burgeoned new evidence of G × E (e.g., Martel et al., 2011; Sheese, Veeker, Rothbart, & Pasner, 2007). More recently, scholars have acknowledged that “vulnerable” individuals might even do better than those without such a vulnerability under supportive conditions (Belsky et al., 2007, Belsky & Pluess, 2009, 2013; Ellis et al., 2011).

Indeed, an extant body of research demonstrated interactions of the DRD4 gene and DRD2 TaqI variant with maladaptive parenting in the development of antisocial behavior (e.g., Beaver, Gibson, DeLisi, Vaughn, & Wright, 2012; Dmitrieva, Chen, Greenberger, Ogunseitan, & Ding, 2011; Martel et al., 2011; Sheese et al., 2007, Waldman, 2007, Zohsel et al., 2014). To illustrate, Bakermans-Kranenburg and Van Izendoorn (2006) found that exposure to low maternal sensitivity exacerbated child externalizing behavior but only in carriers of the DRD4 7-repeat allele. Relatedly, they also found that exposure to high maternal sensitivity decreased externalizing behavior but again only in children carrying the 7-repeat allele. Focusing on children's dysfunction, Sheese and colleagues (2007) demonstrated that lower quality parenting was related to higher levels of sensation seeking, but only for children with the DRD4 7-repeat allele. Also, the work of DeLisi, Beaver, Vaughn, and Wright (2009) revealed that having a criminal parent—placing children in a maladaptive parenting environment conducive to offending—was related to higher levels of serious and violent delinquency in African American children, but only for those carrying the A1 allele.

Studies also found that high psychological control (i.e., parents' manipulative strategies in order to control adolescents' behavior) and low support (i.e., parents' lack of encouragement in the face of failures) are aversive conditions consistently related to the development of adolescent delinquent behavior (e.g., Hoee et al., 2009; Steinberg et al., 1994), and that specifically adolescents carrying a specific DRD4 or DRD2 TaqI variant—linked to blunted dopaminergic functioning, suboptimal physiological arousal, and less intense feelings of pleasure—may experience such maladaptive parenting as highly disappointing (Schultz, 2002). From a diathesis-stress perspective it might be that specifically these adolescents are more vulnerable to adverse effects of maladaptive parenting experiences than others due to their “risky genes” and altered dopamine availability in the brain (Belsky & Pluess, 2013). As a consequence, these adolescents might be at increased risk of getting stuck in coercive cycles with their parents, which are strongly predictive of deviant peer associations.
that, in turn, may lead to a higher likelihood that adolescents will develop delinquent behavior (Patterson & Yoerger, 2002).

Although many G × E findings have been reported over the last decade, there are concerns about whether these findings really constitute evidence for G × E. Specifically, in longitudinal designs alternative explanations for G × E findings cannot completely be accounted for. It may very well be that parents with a specific genetic variant of the DRD4 or DRD2 TaqI have an increased probability to use maladaptive parenting strategies because they have a genetic disposition toward anger or impulsivity, and in such a way transmit a genetic risk for externalizing behavior on to their children (i.e., passive G×E). Thus, parents’ genotype might affect the relation between parenting and adolescents’ behavior and these effects might genetically mediate the G × E between risk exposure (i.e., maladaptive parenting) and problem behavior (i.e., adolescents’ delinquency). To our knowledge, the existing (longitudinal) research has not accounted for such possible effects of parents’ genotypes. We made a first attempt to do so by adopting the Keller (2014) approach. Keller argued that to properly control for effects that might cause spurious observed interactions, one should not only test the main effects of such a covariate but also include interactions of Covariate × Gene and Covariate × Environmental Effects. We used this approach as a way of expanding the search for possible G × E effects in observed G × E by accounting for possible passive genetic effects. More specifically, we accounted for main effects of parents’ genotype and for what we call passive G × E: Parents’ Genotype × Parenting and Parents’ Genotype × Adolescents’ Genotype. What makes this approach distinct relative to the traditional approach is that here we test whether perceived maladaptive parenting could be part of a different, more pathogenic, constellation of parenting behaviors related to parents’ genotype. This relation could give rise to passive G × E effects in which parents’ genotype is a stronger moderator of parenting effects than is adolescents’ genotype (i.e., Passive Parents’ Genotype × Environment) or in which parents’ genotype might moderate the effect adolescents’ genotype may have on delinquent behavior (i.e., Passive Parents’ Genotype × Adolescents’ Genotype).

The aim of the current study was to examine G × E interactions of the DRD4 and DRD2 TaqI variants with two maladaptive parenting styles (high psychological control and low support) in predicting the development of adolescent delinquency. We expected that for DRD4 7-repeat carriers high perceived psychological control and low support would be more strongly related to the presence and development of delinquent behavior, than for those without such an allele. Because of inconsistent effects in the DRD2 literature we explored whether either the DRD2 TaqI A1 or A2 variant was associated with higher risk for the presence and development of delinquent behavior in the light of high perceived psychological control and low support. In addition, we examined G × E more thoroughly by accounting for possible passive genetic effects (i.e., parents’ genotype, Parents’ Genotype × Adolescents’ Genotype, and Parents’ Genotype × Parenting) that might provide an alternative account of observed moderating effects of adolescents’ genotype.

Methods

Participants and procedure

Data for the present study were derived from the 5-wave longitudinal Dutch survey study Family and Health, which investigates family processes in relation to various health behaviors in adolescence (e.g., Harakeh, Scholte, De Vries, & Engels, 2005). Addresses of families with at least two adolescents, aged 13 to 16 years, were derived from registers of 22 municipalities. A letter was sent to all these families inviting them to participate in the longitudinal study. 885 families responded that they were willing to participate and gave their informed consent. These families were telephoned to make sure they fulfilled the entry criteria: parents were married or living together, all family members were biologically related to each other, and participating siblings were neither twins nor mentally or physically disabled. Of the 765 families that fulfilled these criteria, 428 families were selected to ensure an equal distribution of adolescent educational level and an equal number of all the possible sibling dyads (i.e., boy–boy, girl–boy, boy–girl, girl–girl). Chi-square statistics showed no significant difference between included (n = 428) and excluded (n = 337) families with regard to educational level for either father or mother (ps > .05).

The present study used data of the youngest adolescent in each family because patterns of delinquency underlie an age-crime curve, which tends to peak between early to mid-adolescence. Of the 428 included families, 311 families agreed to be genotyped; three adolescents could not be genotyped. The final sample consisted of 308 families who provided us with full information across all five waves. Attrition analyses were performed to investigate whether families who gave their consent for genotyping and took part at all waves (participants: N = 308) differed from those who did not (dropouts: n = 120). T tests showed that participants and dropouts did not significantly differ in terms of delinquent behavior, psychological control, support, and age (ps > .05). Also, chi-square statistics showed no significant difference with regard to educational level and sex.

Data collection of Wave 1 took place in the winter of 2002 to 2003, with Waves 2 through 5 taking place after 1, 2, 3, and 4 years, respectively. A trained interviewer visited participating families at their homes. In the presence of the interviewer, all family members individually completed extensive questionnaires, which took approximately two hours. Family members were not allowed to discuss questions with each other. When all family members had completed the questionnaires, the family received €30 ($33.00) at each wave. At Wave 4, DNA samples were collected by means of saliva.

At Time 1 (T1) the mean age of mothers and fathers was 43.9 (SD = 3.59) and 46.2 (SD = 3.97), respectively. Parents were relatively highly educated. Of the mothers, 38.9% followed higher education (i.e., university of applied science—also known as higher
vocational education—and university of science), 24.4% intermediate education (i.e., intermediate vocational education within vocational schools that prepares people to a specific trade), and 36.7% lower education (i.e., elementary school 2.6%; high school 34.1%), of the fathers, 51.3%, 22.8%, and 25.9% (i.e., elementary school 17%; high school 24.2%), respectively. The mean age (T1) of participating adolescents was 13.4 (SD = 1.1) of whom 47% were boys. The range of age for the younger siblings at the successive waves was 12 to 14 years (T1), 13 to 15 years (T2), 14 to 16 years (T3), 15 to 17 years (T4), and 16 to 18 years (T5). A small group of adolescents was not born in The Netherlands (< 4%). Roughly one third (33.3%) attended lower education (i.e., vocational or technical secondary education), one third (36.5%) attended intermediate general education, and one third (30.2%) attended the highest education level in secondary school (i.e., pre-university education). There were no dropouts from high school. Explicit approval for the data collection was obtained from Central Committee on Research involving Human Subjects in The Netherlands.

**Measures**

**Delinquent behavior**

Delinquency was measured with a Dutch questionnaire that specifies frequencies of participation in criminal acts by adolescents (Houtzager & Baerveldt, 1999). This measurement was chosen because it is widely used in The Netherlands and because it includes most frequent delinquent behaviors within this age group (Nijhof, Scholte, Overbeek, & Engels, 2010). Specifically, adolescents were asked whether they engaged in 13 rule-breaking activities in the past 12 months (e.g., ‘Have you ever set fire to a building?’; ‘Have you ever participated in a serious physical fight?’; ‘Have you ever stolen a moped or a scooter?’; ‘Have you ever seriously beaten someone with an object or injured someone?’) at each wave. These 13 items were scored on a four-point Likert scale (1 = never; 2 = once; 3 = two or three times; and 4 = four or more times). The distribution of delinquency was relatively low in absolute terms and highly skewed. Therefore, in line with prior research (e.g., Caspi & Moffitt, 1991; Overbeek, Vollebergh, Meeus, Engels, & Luijpers, 2001; Platigorsky & Hinshaw, 2004), each item was dichotomized as 0 (never happened) and 1 (happened at least once). The dichotomized items were then summed to create a scale describing the number of delinquent acts perpetrated at each time point. Cronbach’s alphas were 0.65, 0.83, 0.88, 0.85, and 0.74, respectively.

**Parenting**

Parenting was rated by adolescents for parents separately at T1. The support scale of parenting was assessed with a subscale of the Relational Support Inventory (Scholte, Van Lieshout, & Van Aken, 2001). The 12-item questionare (e.g., ‘My mother shows me that she loves me’ and ‘My mother supports me in the things I do’) was rated on a response scale, ranging from 1 (very untrue) to 5 (very true). A Dutch translation of the 48-base pair direct repeat polymorphism (VNTR) in **DRD4** was genotyped by amplifying 10 ng of genomic DNA in a 20-µl volume with the following components: 0.05 µmol/L of fluorescently labeled forward primer VIC-5’-GCGACTACGGGGTCTACCTCG-3’ (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands), reverse primer 5’-AGGCCCTCTATGGCCCTG-3’, 0.4 mM of deoxynucleoside triphosphates (dNTPs), and 0.5 U of Taq polymerase (Takara, Lonza Verviers S.p.r.l., Verviers, Belgium). These were in a GC buffer (Takara, Lonza Verviers S.p.r.l.) with 1 M betaine. The cycling conditions for amplification involved 1 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, with an additional 5 min at 72°C. The length of the alleles was determined by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands). Hardy-Weinberg equilibrium (HWE) proportions were estimated and no deviations from these proportions were found in adolescents, fathers, or mothers (p = 0.02–0.90). The **DRD2** genotype was dummy-coded as 0 (no 7-repeat allele) and 1 (at least one 7-allele). We followed the same procedure to genotype the **DRD4** gene of both mothers and fathers.

### **DRD4 genotyping**

The Taq DNA polymerase (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands) was used. Genotyping was carried out in a volume of 10 µl containing 10 ng of genomic DNA, 0.4 U of Taqman Mastermix (Applied Biosystems), 12.5 µL of the Taq polymerase, and 3.875 µl of H2O. Samples were run on a 7500 Fast-Real-Time PCR System and genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems). To investigate the random genotyping error rate, the lab included five replicate DNA samples per 96-well plate, which were 100% consistent. In addition, four blanks were included in each plate, which were required to be negative. By running PEDCHECK (O’Connell & Weeks, 1998) for single-point Mendelian inconsistencies on the markers, we identified one family with potential pedigree errors. This family was removed from the analysis. **HWE** proportions were estimated from parental genotype information.
using the Markov chain Monte Carlo approximation of the exact test implemented in the GENEPOP package V3.3 (Raymond & Rousset, 1995). No deviations from HWE were detected for either adolescents, fathers, and mother (ps > .05). The DRD2 genotype was dummy-coded into 0 (A2A2) and 1 (A1A2 and A1A1). We followed the same procedure to genotype the DRD2 gene of both mothers and fathers.

Analytic strategy

Latent growth curve modeling (LGCM) was used in Mplus (Muthén & Muthén, 2008-2015). As individual growth is estimated for each adolescent separately, LGCM is an excellent way to take individual variation in the development of delinquent behavior into account and to investigate whether certain predictors are related with differential developmental patterns. As delinquency was not normally distributed, the parameters in the models were estimated by applying the maximum likelihood estimator with robust standard errors that corrects for a non-normal distribution of the dependent variable.

First, we specified a basic developmental model estimating an intercept (i.e., initial level), linear slope (i.e., mean change across one year time intervals), and quadratic slope (i.e., mean change of the slope parameter across time intervals) of delinquent behavior. Building on this basic developmental model, we entered variables that permitted a deeper understanding of the change processes of the G × E of interest. In total, we tested the following four LGCM models: (a) DRD4 × Psychological Control, (b) DRD4 × Parental Support, (c) DRD2 × Psychological Control, and (d) DRD2 × Parental Support. In the simple models, we included adolescents’ genes, parenting, and the interaction between adolescents’ genes and parenting in the analyses. In the advanced models, we additionally included single effects of parents’ genes and the passive G × E between parents’ genes and parenting and between parents’ genes and adolescents’ genes as variables in the analyses. These passive genetic effects were entered in either the DRD4 interaction or DRD2 interaction model (see Table 2 and 3). To avoid multicollinearity, variables were centered before computing the interaction terms. Model fit is considered adequate if the root mean square error of approximation (RMSEA) is < .05 and comparative fit index (CFI) values is > .95 (Hu & Bentler, 1999). If χ² df all, the CFI and RMSEA are set to 1.00 and < 0.01, respectively, constituting a normed fit index. In that case, it is sufficient to check the p-value of the chi-square test of model fit. A good fit is present when the p-value is not significant (Van de Schoot, Lugtig, & Hox, 2012).

Table 1. Correlations Between DRD4/DRD2 Genes, Perceived Parental Psychological Control and Support, and Delinquent Behavior at Waves 1-5

<table>
<thead>
<tr>
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<th>1.</th>
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Note. DRD4 = dopamine D4 receptor gene, 0 = both alleles shorter than 7-repeat allele, 1 = at least one 7-repeat allele; DRD2 = dopamine D2 receptor; 0 = A2A2, 1 = A1 and A1A2; a Point biserial correlations. *p < .05. **p < .01.

Effect sizes (i.e., R²) were derived by comparing the residual error variances across models plus the deviance (Hox, 2010). The basic developmental model of delinquent behavior was used as a baseline model to examine effect sizes of the main effects. This because the basic developmental model did not introduce any explanatory variables (except intercept, linear slope, and quadratic slope) and decomposes the total variance of delinquent behavior over time. Because there are no explanatory predictors in the model, the total variance of delinquent behavior is equivalent to the total error variance. To examine effect sizes of G × E, the residual error variance of the main effect model was used as a baseline model to which the residual variance of the G × E was compared with.

Results

Descriptive statistics

Of the 308 adolescents studied, 108 (35.1%) carried at least one DRD4 7-repeat allele. For the DRD2 gene, a total of 205 adolescents (66.3%) were A2 homozygous, thus with 104 (33.7%) being heterozygous or A1 homozygous. Of the mothers and fathers studied, 36.3% and 36.0% carried a DRD4 7-repeat allele and 34.3% and 29.8% were A2 homozygous, respectively. Means of psychological control and support were 2.20 (.50) and 4.01 (.93), respectively. Percentage of engaging in one or more rule-breaking activities—among all participants (i.e., basic developmental model)—ranged from 29.9% to 44.8%. The distribution of delinquent behavior was comparable with other studies on non-clinical samples (e.g., Harden et al., 2012).

Correlations among model variables are illustrated in Table 1. The DRD4 and DRD2 genes of both adolescents and parents were not correlated significantly with
delinquent behavior. Perceived psychological control at T1 was significantly correlated with higher delinquent behavior at most waves, whereas perceived support at T1 was significantly correlated with lower delinquent behavior at most waves. Also, the point biserial correlation between DRD4 gene and adolescents’ self-reports of psychological control and support was significant, showing that adolescents with the DRD4 7-repeat allele reported more psychological control and less parental support than adolescents without the DRD4 7-repeat allele. Parents’ DRD4 or DRD2 genes were not correlated with parenting or adolescent delinquency.

LGCM results

Development of delinquent behavior

Results showed that a linear model did not fit the data well, $\chi^2 (N = 308, df = 10) = 48.28, p < .001$ (CFI = .71, and RMSEA = .11). To improve fit, we specified a model including a quadratic growth parameter. This model fit the data well, $\chi^2 (N = 308, df = 6) = 5.26, p = .51$ (CFI = 1.00, and RMSEA < .001). The estimates for all parameters were significant, meaning that delinquency rates differed significantly from zero. The intercept estimate demonstrated the mean of delinquency at baseline ($\beta = .99, p < .001$). The linear slope estimate ($\beta = .025, p = .014$) demonstrated that delinquency increased over time. The quadratic slope estimate ($\beta = -.08, p < .001$) demonstrated that delinquency increased across early and mid-adolescence but then decreased across late adolescence (see Tables 2 and 3).

**Table 2. Outcomes of Latent Growth Curve Modeling regarding Gene by Environment Interactions in Delinquent Behavior Involving DRD4 Gene and Perceived Psychological Control**

Note. Means of the initial developmental model are presented; df = degrees of freedom; DRD4 = dopamine D4 receptor gene; 0 = both alleles shorter than 7-repeat allele, 1 = at least one 7-repeat allele; CFI = comparative fit index; RMSEA = root mean square error of approximation. As $\chi^2 < df$, the CFI is set to 10 and RMSEA to <.001, which makes it sufficient to read off whether the p value is not significant. *p < .05. **p < .01. ***p <.001.

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<th>Intercept</th>
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<th>Quadratic</th>
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<td>0.16</td>
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<tr>
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<tr>
<td>Parent × Gene</td>
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**DRD4 × Psychological Control**

In a simple model, we found that the DRD4 was not related to the intercept ($\beta = .00, p = .940, R^2 = .00$) or slopes ($\beta = .05, p = .461, R^2 = .00; \beta = .05, p = .461, R^2 = .00$) of adolescents’ delinquency. Psychological control was not significantly related to the intercept ($\beta = .12, p = .070, R^2 = .03$) and linear slope ($\beta = .14, p = .057, R^2 = .03$) but was significantly negatively related to the quadratic slope ($\beta = -.17, p = .015, R^2 = .04$), indicating that adolescents perceiving higher levels of psychological control did not show higher levels of delinquent behavior at intercept or a steeper increase of delinquent behavior across early and mid-adolescence, but did show the highest decrease across late adolescence. Also, we found a significant interaction between DRD4 gene and psychological control at intercept ($\beta = .18, p = .041, R^2 = .06$); there was no relation between psychological control and delinquent behavior for those carrying two short alleles of the DRD4 gene ($R^2 = .00$), but higher psychological control was significantly related to higher levels of delinquent behavior for those carrying at least one DRD4 7-repeat allele ($R^2 = .08$). However, in an advanced model—after DRD4 mother, DRD4 father, DRD4 Mother × Psychological Control, DRD4 Father × Psychological Control, DRD4 Mother × DRD4 Gene Adolescent, and DRD4 Gene Father × DRD4 Adolescent were entered in the analysis—no significant interaction emerged between DRD4 gene and psychological control at intercept, $\chi^2 (26) = 32.80$. 

**DRD2 × Parental Support**

In a simple model, we found that the DRD2 gene was not related to the intercept ($\beta = .08, p = .163, R^2 = .00$). The DRD2 gene was, however, negatively related to the linear slope ($\beta = -.13, p = .011, R^2 = .01$) and positively related to the quadratic slope ($\beta = .12, p = .017, R^2 = .01$), indicating that adolescents with the A2A2 genotype showed a steeper increase of delinquent behavior.
in early and mid-adolescence, but the highest decrease across late adolescence. Parental support was negatively related to the intercept ($\beta = -0.20$, $p = .001$, $R^2 = .05$) but not to the linear and quadratic slopes ($\beta = .00$, $p = .958$, $R^2 = .01$, $\beta = .04$, $p = .588$, $R^2 = .01$), indicating that adolescents reported higher levels of delinquent behavior when perceiving lower levels of parental support at baseline. Also, we found a significant interaction between DRD2 gene and parental support. The interaction was positively related to the linear slope ($\beta = 1.17$, $p = .034$, $R^2 = .02$) and negatively related to the quadratic slope ($\beta = -1.27$, $p = .029$, $R^2 = .02$); there was no relation between low and high support and delinquent behavior for those carrying at least one A1 allele (linear slope: $R^2 = .01$, quadratic slope: $R^2 = .00$). However, those with the A3A2 genotype reported a significant steeper increase in delinquent behavior across early and mid-adolescence when perceiving low parental support (linear slope: $R^2 = .04$), but also showed a significantly stronger decrease in levels of delinquent behavior across late adolescence when perceiving low support (quadratic slope: $R^2 = .05$).

In an advanced model, we found that the DRD2 main effects disappeared after entering DRD2 mother, DRD2 father, DRD2 Mother × Psychological Control, DRD2 Father × Psychological Control, DRD2 Mother × DRD2 Adolescent, and DRD2 Father × DRD2 Adolescent in the regression model, $\chi^2 (24) = 21.63$ (linear slope: $\beta = -0.13$, $p = .375$, $R^2 = 0.00$, quadratic slope: $\beta = 0.12$, $p = .572$, $R^2 = 0.00$; see Table 3). However, the interaction effects remained significant for both slopes (linear slope: $\beta = 0.21$, $p = .013$, $R^2 = 0.02$, quadratic slope: $\beta = 0.21$, $p = .014$, $R^2 = 0.02$) with interpretations of results being the same (see Figure 1). This indicates that no passive G × E effects emerged and thus the observed interaction of DRD2 × Parental Support survived as significant. Notably, the effect sizes between support and delinquent behavior were also maintained for those carrying the DRD2 A2A2 genotype (linear slope: $R^2 = 0.04$, quadratic slope: $R^2 = 0.05$). The interactions between DRD4 gene and parental support and DRD2 gene and psychological control were not significant, neither in a simple nor advanced model.

**Discussion**

This study tested whether the DRD4 and DRD2 genes interacted with adolescent-reported psychological control and parental support in predicting development of delinquent behavior in a general population sample of early adolescents. After accounting for passive genetic effects (i.e., parents’ genotype, Parents’ Genotype × Adolescents’ Genotype, and Parents’ Genotype × Parenting), results from LGCM indicated that the DRD4 7-repeat allele and DRD2 A2A2 genotype were not related to adolescents’ delinquent behavior. As parents are likely to transmit genes that might promote oppositional and rule-breaking behavior onto their children, it is plausible that parents’ genes explained a part of the associations between adolescents’ genes and their delinquent behavior. However, results did reveal a significant interaction between DRD2 and parental support, indicating that adolescents with the DRD2 A2A2 genotype were more vulnerable for maladaptive parenting, developing more delinquent behavior as a consequence. No significant interactions emerged for DRD4 with parental support and psychological control, nor for DRD2 with parental psychological control.

It is interesting to note that the point biserial correlation between DRD4 and adolescents’ self-reports of their parents’ psychological control and support was significant, showing that adolescents with the DRD4 7-repeat allele reported more psychological control and less parental support than adolescents without the DRD4 7-repeat allele. This could be an indication of perceptual GE, in that adolescents with such an allele were inclined to experience less support and more psychological control than those without such an allele. Results further showed that, after again accounting for passive genetic effects, DRD4 7-repeat and DRD2 A2A2 carriers were not at

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</tr>
<tr>
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<tr>
<td>Quadratic slope</td>
<td>-0.17 (0.08)*</td>
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**Table 3. Outcomes of Latent Growth Curve Modeling regarding Gene by Environment Interactions in Delinquent Behavior Involving DRD2 Gene and Perceived Parental Support**

Note: Means of the initial developmental model are presented. df = degree of freedom; DRD2 = dopamine D2 receptor gene; 0 = A2A2, 1 = A1A1 and A1/A2; CFI = comparative fit index; RMSEA = root mean square error of Approximation. As $\chi^2 < df$, the CFI is set to 1.0 and RMSEA to <.001, which makes it sufficient to read off whether the $p$ value is not significant. *$p < .05$. **$p < .01$. ***$p < .001$. 

**Figure 1.** A hypothetical model of interaction between DRD2 and adolescent's delinquent behavior.
Development of delinquent behavior

Figure 1. Development of delinquent behavior (as indicated by mean scores) across five yearly waves with low (−) or high (+) perceived parental support for adolescents with (+ A1) or without (− A1) the dopamine D2 receptor (DRD2) A1 allele, after accounting for passive genetic effects in the advanced model. With significant differences at Wave 2, Wave 3, and Wave 4.

higher risk for the presence and development of delinquency in adolescence. This is not surprising, as it is well-known that direct associations between genes and complex phenotypes such as delinquency are unlikely (Rutter et al., 1997) and we accounted for heritability of "risky genes.”

Interaction effect of DRD2 × Parental Support

We found a G × E interaction between DRD2 gene and parental support in the development of delinquent behavior, implying that especially adolescents with the A2A2 genotype who experienced low levels of support showed a stronger increase in delinquent behavior across early and mid-adolescence but a steeper decrease across late adolescence, compared with the other three groups. After accounting for passive genetic effects, the DRD4 × Psychological Control interaction was no longer significant. Although we found no credible evidence that DRD4 7-repeat carriers were more vulnerable for high psychological control, none of the passive genetic effects itself significantly predicted adolescents’ delinquent behavior (except for one association with the quadratic component of the model, Table 2). Thus, the changes in observed G × E estimates were probably not explained by passive G × E. Needless to say, replication is important to confirm the present evidence.

With regard to the maintained DRD2 × Parental Support interaction (see Figure 1), we see a clear age-crime curve. Delinquent behavior increases with age until adolescents reach mid-adolescence and then decreases with age (Farrington, 1986). However, this developmental trend was only observed among A2A2 carriers that perceived low parental support. Specifically, those adolescents reporting lower levels of parental support did not show higher levels of delinquency at early and late adolescence (i.e., Wave 1 and Wave 5), but did show a steeper increase in delinquency during the transition from early to mid-adolescence (i.e., Waves 2 through 4). However, this age-crime curve does not explain why specifically this subgroup of adolescents was more vulnerable for low perceived parental support during this period.

There have been mixed results regarding the functional significance of the A1 and A2 allele. Although most studies specically related the Taq A1 allele to decreased receptor density in the brain (e.g., Pohjalainen et al., 1998), others did not (Laruelle, Gelernter, & Innis, 1998). Therefore, it may be that an altered dopaminergic function linked to a specific allele is not the straightforward reason why adolescents carrying a certain DRD2 genotype would be more likely to be vulnerable for low support. Specially, the Taq A1 allele in the DRD2 gene is functionally related to another gene nearby the DRD2 gene (i.e., ANKK1 gene). The ANKK1 and DRD2 genes may be co-actors in a genetic haplotype (i.e., a complex of functionally related genes) that affects the functioning of dopaminergic neurotransmitter pathways (Ponce et al., 2009). We recommend that future research should not focus only on the interpretability of G × E results in the light of passive genetic effects, but also in the light of more complex indices of genetic functioning such as haplotypes (see Dick, Latendresse, & Riley, 2011).

Effect sizes of gene by environment interactions

The effect sizes in the present study (i.e., R²) are clearly very modest. Before passive genetic effect were trimmed from the models, the effect sizes for main effects of genes were not detectable (i.e., DRD4) or accounted at most for only 1% in the development of delinquency behavior over time (i.e., DRD2). The observed effect sizes for the interaction effects between genes and maladaptive parenting were also small. When examining effect size differences for parenting on the development of delinquency across genotypes, effect sizes increased among those most genetically vulnerable for maladaptive parenting. Psychological control accounted for 8% in delinquency among those most vulnerable for high psychological control (i.e., DRD4 7-repeat: intercept) and parental support for 4% and 5% among those most vulnerable for low parental support (i.e., DRD2 A2A2: linear and quadratic slope, respectively). After passive genetic effects were trimmed from the models, no effect sizes for main effects were detected. The effect sizes for the interaction effects were respectively 0.02 and 0.04 and 0.05 among those most genetically vulnerable for maladaptive parenting.

It is well-known that the explained variance of genetic main effects in the development of psychopathology is very low or even absent (Manolio et al., 2009; Risch & Merikangas, 1996). This is not surprising because genes might not be directly related
to psychopathology but may lead to increased risk in interaction with specific envi-
ronmental factors (Rutter, 2012). Effect sizes of $G \times E$ are, however, often not much
larger (Mechanic & Hutter, 2015; Rutter, Moffitt, & Caspi, 2006) as was also evident
from our present analyses. Testing $G \times E$ involves measuring how much behavioral
variation is attributed to the interaction and will therefore be subject to a lot of
noise. However, some noise might have a plausible reason to account for addition-
al behavioral variance. In the present study, we specifically accounted for passive
$G \times E$. This resulted in more accurate effect sizes of observed $G \times E$ but also in evidence
that passive genetic effects might additionally account for variance in outcomes. It is
important that further research takes into account this possibility. Issues surrounding
the correlation between genetic and environmental variables, restriction of range, and
an overall limited variance in measurement of environmental factors have been identi-
fied as main causes of the difficulty to detect and replicate $G \times E$ (e.g., Dick et al., 2011;
Duncan & Keller, 2014). All these issues should be considered carefully in interpreting
$G \times E$ evidence. What is needed in future replication efforts for the present findings
are large sample sizes, experimental designs, and well operationalized constructs.
This will minimize the risk of coming up with Type I errors (i.e., false positives).

The evidence for $G \times E$ underscores the diathesis-stress model (Zuckerman, 1999).
This is consistent with numerous previous studies that found $G \times E$ related to child’s
dysfunction (e.g., Caspi, Harin, Holmes, Uher, & Moffitt, 2011; Caspi et al., 2002; Sheese
et al., 2007). Although there is evidence that children with “risky genes” will also benefit
most from environmental enrichment, here we found that adolescents with a specific
genetic disposition are more likely to do worse under adversity. However, in future
studies, a stringent test of the differential-susceptibility hypothesis (Belsky et al.,
2007, Belsky & Pluess, 2009, 2015, Boyce & Ellis, 2005) could be made by measuring
adolescents’ functioning along a continuum from dysfunction to competence rather
than from dysfunction to its absence (Belsky & Pluess, 2009) or by using experimental
designs to test variation in response to positive experiences (Bakermans-Kranenburg

An important observation is the fact that the hypothesized $G \times E$ interactions were
not systematically found across the models tested. Perhaps, this has something
to do with statistical power that remains a problem in longitudinal $G \times E$ studies.
More stringent replications in large-scale prospective or experimental studies are
therefore recommended. This is especially relevant in light of the fact that almost
all originally published candidate based $G \times E$ results in the field of psychiatry are
significant (45/47, 96%) but less than one third of the replication attempts is (10
out of 37, 27%; Duncan & Keller, 2011). This may represent a publication bias toward
significant, perhaps less stringently controlled, results. In light of this possible publi-
cation bias, reporting both models with and without additional “control” variables is
crucial, to make transparent the extent to which a result is accounted by (or robust
against) such a control effect (Simmons, Nelson, & Simonsohn, 2011). An important
related point here is that we conducted four separate tests of $G \times E$ (i.e., $DRD4 \times
Psychological Control, $DRD4 \times Parental Support, $DRD2 \times Psychological Control, $DRD2
\times Parental Support), with two opportunities (i.e., simple model, advanced model) for
significant effects in each case. Accordingly, a traditional Bonferroni correction for
multiple testing would have resulted in non-significant effects for all $G \times E$ findings,
stipulating the need for replication of the patterns here.

Limitations and strengths

Some limitations have to be mentioned. First, most adolescents were from an indig-
eous Dutch background and lived with both parents. Therefore, our results may not
be general to populations that are more heterogeneous. Then again, with regard to
genetic stratification it is better to have a homogeneous ethnic group, as was the case
in our sample. Second, both parenting as well as delinquent behavior were assessed
by adolescent-reports. It might be that perceived parenting is not a measure of an
environmental factor, but may in part also measure a child characteristic. However,
the data showed significant correlations between parents’ reports and adolescents’
self-reports on parenting, indicating that adolescents’ self-reports of parenting
converged with those of parents themselves. In the present analyses, we examined
the way adolescents perceived parenting, as this has been argued to be pivotal,
adolescents’ perceptions of parent behaviors will determine their impact (Steinberg
et al., 1994). In addition, it is well-known that parents’ reports on parenting practices
are vulnerable to social desirability response bias and therefore are more likely to outline
the positive characteristics of families (Steinberg, 2001). As values of the parenting
styles were relatively stable over time we did not investigate whether the impact
of parenting changed as a function of developmental stage. However, it could be
interesting for future research to also investigate such changes in parenting over time.
Also, further research could benefit from including earlier assessments of parenting
to explain the development of delinquency observed at baseline. Furthermore, we
must acknowledge that complex phenotypes such as delinquent behavior may have
multifactorial polygenic etiologies (i.e., polygenic or multi-genic effects). Although
we followed strategic steps (e.g., providing a biological rationale, and working with
a prospective design and established environmental risk measurement) to organize
a high quality single candidate study (Moffitt, Caspi, & Rutter, 2005), this approach
carries limited information about the overall variation within and between co-act-
ing genes. Therefore, future research should also focus on more complex indices of
genetic functioning (Dick et al., 2011), because currently high-throughput genotyping
technologies are available to test such complex multifactorial polygenic etiologies.

Chief among the strengths of the present study are the genetically informed longi-
tudinal design with its adequate sample size and the sophisticated analytic strategy.
We longitudinally investigated $G \times E$ interactions of the $DRD4$ and $DRD2$ variants with
psychological control and parental support in the development of delinquent behavior
and accounted for passive genetic effects by adopting a new approach using the
Keller (2014) proposal. We demonstrated that passive $G \times E$ were negligible but that
the observed G × E only survived in case of the DRD2 × Parental Support interaction. Also, we demonstrated that passive genetic effects accounted for additional variance in delinquent behavior.

Conclusion

The present study demonstrates that adolescents with the DRD2 A2A2 genotype were more vulnerable for low parental support, developing more delinquent behavior as a consequence. What makes this study especially distinct relative to other longitudinal G × E studies is that rather than focusing only on G × E, we adopted a new approach of accounting for parents’ genotypes and passive G × E. Although the DRD2 × Parental Support interaction survived as significant, its observed effect size was relatively modest. Replication studies are thus needed to confirm the present evidence that maladaptive parenting is more strongly related to delinquency in adolescents carrying the DRD2 A2A2 genotype.
ORCHIDS: An Observational Randomized Controlled Trial on Childhood Differential Susceptibility
Chapter 3

**ORCHIDS: An Observational Randomized Controlled Trial on Childhood Differential Susceptibility**

**KEYWORDS**
- Randomized controlled trial
- Externalizing behavior
- Parenting
- Gene-environment interaction
- Differential susceptibility

**PRESS**

¹The first two authors contributed equally to this work

**ABSTRACT**

**Background:** A central tenet in developmental psychopathology is that childhood rearing experiences have a major impact on children's development. Recently, candidate genes have been identified that may cause children to be differentially susceptible to these experiences (i.e., susceptibility genes). However, our understanding of the differential impact of parenting is limited at best. Specifically, more experimental research is needed. The ORCHIDS study will investigate gene × (gene ×) environment interactions to obtain more insight into a) moderating effects of polymorphisms on the link between parenting and child behavior, and b) behavioral mechanisms that underlie these gene × (gene ×) environment interactions in an experimental design.

**Methods/design:** The ORCHIDS study is a randomized controlled trial, in which the environment will be manipulated with an intervention (i.e., Incredible Years parent training). In a screening, families with children aged 4–8 who show mild to (sub)clinical behavioral problems will be targeted through community records via two Dutch regional healthcare organizations. Assessments in both the intervention and control condition will be conducted at baseline (i.e., pretest), after 6 months (i.e., posttest), and after 10 months (i.e., follow-up).

**Discussion:** This study protocol describes the design of a randomized controlled trial that investigates gene × (gene ×) environment interactions in the development of child behavior. Two hypotheses will be tested. First, we expect that children in the intervention condition who carry one or more susceptibility polymorphisms will show significantly lower levels of problem behavior and higher levels of prosocial behavior after their parent(s) received the Incredible Years training, compared to children without these polymorphisms, or children in the control group. Second, we expect that children carrying one or more susceptibility polymorphisms will show a heightened sensitivity to changes in parenting behaviors, and will manifest higher emotional synchronization in dyadic interchanges with their parents. This may lead to either more prosocial behavior or antisocial behavior depending on their parents' behavior.

**Trial registration:** Dutch Trial Register (NTR3594).
A central tenet in developmental psychopathology is that childhood rearing experiences have a major impact on children's development across life (Schaffer, 2000). At the same time, we know that not all children are equally susceptible to these experiences (Klein Velderman, Bakermans-Kranenburg, Juffer, & Van Uzendoorn, 2006). Grounded in a diathesis-stress model, there has been growing attention for research on individuals' genetic susceptibility to parenting. The diathesis-stress model holds that some children, due to a specific vulnerability, are more likely to be negatively affected by environmental risk, such as parental harshness, than others (Caspi et al., 2002; Dick et al., 2011; Kochanska, Philibert, & Barry, 2009). A typical characteristic of these studies is that they only examined environmental adversity and negative child outcomes. It may therefore be that we, for a long time, only studied so-called dandelions; the resilient children that do well even in the face of severe adversity. In doing so, we may have overlooked the orchids; children who will suffer severely if ignored or maltreated, but flourish spectacularly when receiving adequate care. This metaphor forms the basis of an intriguing alternative hypothesis, namely the differential susceptibility hypothesis which holds that some children, due to a specific susceptibility factor, are more likely to be affected by environmental factors, for better and for worse (Belsky et al., 2007, Belsky & Pluess, 2009, Boyce & Ellis, 2005).

Preliminary evidence for this differential susceptibility hypothesis has accumulated over the past years. Previous studies demonstrated, for example, that children with the dopamine receptor D4 (DRD4) 7-repeat allele showed significantly more externalizing problem behavior when mothers were insensitive, but also showed less problem behavior when mothers were highly sensitive, compared to those without the DRD4 7-repeat allele (Bakermans-Kranenburg, Van Uzendoorn, Pijman, Mesman, & Juffer, 2008). Studies have identified several candidate genes underlying children's differential susceptibility (e.g., monoamine oxidase A (MAOA) gene, dopamine transporter (DAT) gene, dopamine receptor D4 (DRD4) gene, dopamine receptor D2 (DRD2) gene, serotonin transporter (5-HTTLPR) gene, and the catechol-o-methyltransferase (COMT) gene (Cicchetti, Rogosch, & Sturge-Apple, 2007; Kahn, Khoury, Nichols, & Lanphear, 2003, Karayiorgou et al., 1999; Pluess et al., 2011; Van Uzendoorn, Bakermans-Kranenburg, & Mesman, 2008; Waldman, 2007). However, the tenability of the genetic differential susceptibility hypothesis is still unclear for several reasons. First, most previous studies only measured the presence or absence of environmental adversity and developmental problems, but not environmental enrichment and children's competence. The absence of parental maltreatment, however, is not the same as parental warmth or sensitivity (Pluess & Belsky, 2010). Only environmental conditions and outcomes ranging from dysfunction to competence make it possible to avoid ceiling effects in testing differential susceptibility (Belsky & Pluess, 2009, Taylor et al., 2006). Most importantly, most previous studies used correlational designs (Riley, 2008) and therefore alternative explanations for gene by environment (G × E) interactions cannot be ruled out. For example, children with oppositional behavior may be genetically more likely to evoke harsh parental discipline and to actively select environments that support their problem behavior.

Trials in which families are randomly distributed across different environmental conditions offer a solution to this problem, because they permit a manipulation of the environment that is independent of children's genetic makeup and developmental histories (e.g., Overbeek, Weeland, & Chhangur, 2012). To our knowledge four randomized controlled trials on G × E interactions in children's social emotional development have been conducted so far (Bakermans-Kranenburg et al., 2008, Brody, Beach, Philibert, Chen, & Murry, 2009, Cicchetti, Rogosch, & Toth, 2011, Van den Hoofdakker et al., 2012). These pioneering studies delivered important new insights, however their impact suffered from limitations as well. First, although the trials measured environmental enrichment the outcome was usually measured as a decrease in adolescent and child problem behavior, overlooking a possible increase in competent behavior. However, as argued above, to adequately examine differential susceptibility, measurements of both environment and child behavior should range from dysfunction to competence. Second, the trials did not examine the possible underlying behavioral mechanism through which a G × E interaction may lead to different behavioral outcomes. It may be that carriers of candidate susceptibility genes show heightened behavioral reactivity. For example, children with low levels of dopaminergic functioning, associated with low reward sensitivity (Matthys et al., 2013), may improve more during and after parent management training than those with high levels of dopaminergic functioning associated with high reward sensitivity, because they can benefit more from the individualization of use of rewards and extensive praising by parents. Likewise, children with decreased serotonergic functioning, associated with negative affect/mood, may improve strongly during and after parenting training due to the effect of an increase in positive parental emotions on this affect/mood (Matthys et al., 2012). A highly reactive child will likely show an intense, mirroring emotional response to both negative and positive discipline (Kohn, 1991; Strelau, 1893), which, in turn may lead to emotional synchronization in parent–child interactions. This congruency in affect may then lead to the development of either problem or prosocial behavior, depending on either positive or negative interactions with parents. Therefore, research should also investigate genetic expression 'outside the skin' the mechanisms through which genetic variation moderates the impact of environmental influences on individuals' development. A randomized controlled trial can test hypotheses about underlying behavioral processes by examining whether certain mechanisms change in the experimental condition, mediating the intervention effect (Reiss & Leve, 2007).

**Aim and Hypotheses**

The ORCHIDS study is a genetically informed randomized controlled trial to examine possible G × E and G × G × E (i.e., polygenic) interactions in the development of child behavior. The study examines parenting in its full scope, from both harsh and inconsistent to positive, sensitive, and appropriate parenting behavior as well as from children's problem behavior and difficulties to their skills, competencies, and strengths. The primary aim is to investigate whether enrichment of the environment,
based on the Incredible Years (IY) parent training, has more effect on a genetically susceptible subgroup of children, and to investigate why this may be the case. We expect that the parent training will bring about an environmental enrichment, leading to behavior changes in the participating parents. Two hypotheses will be tested. First, we expect differential susceptibility, which means that children in the intervention condition who carry one or more susceptibility polymorphisms (i.e., carrying a DRD2 A1 allele, DRD4 7-repeat allele, DAT 10-repeat allele, MAOA low-activity (short) allele, and or a COMT val allele) will show a significantly higher decrease of problem behavior and increase of prosocial behavior after their parent(s) received the parent training, compared to children without such susceptibility polymorphisms and children in the control group. In the control group, we expect this same genetic subgroup to show most behavior problems and least prosocial behavior. Second, we expect that emotional synchronization in parent–child interactions will mediate the intervention effect. Specifically, we expect that children who carry one or more susceptibility polymorphisms show a higher synchronization to their parents’ affect than children without these susceptibility polymorphisms. Therefore, we expect these children to benefit most from the increase in parental positive affect and sensitivity induced by the Incredible Years intervention.

Methods

Design

The ORCHIDS study is a randomized controlled trial with an intervention (i.e., the Incredible Years parent training) and a control condition that tests gene–based differential susceptibility to changes in parenting. Participants will be 480 families, with children aged 4–8 who show mild to (sub)clinical externalizing behavior problems. Of those families, 160 will be randomly assigned to the intervention condition and 320 families to the control condition. After enrollment in the trial and randomization, the baseline assessment (pretest) will be carried out. The Incredible Years (IY) program will be implemented after these baseline assessments. Participants in the control condition will receive no intervention, but are allowed—and, in case needed, are assisted—to seek mental health care and parenting support through regular services. Posttest and follow-up assessments will be conducted after 6 months and after 10 months, respectively. Approval for data collection was obtained from the central committee on research involving human subjects in The Netherlands (METC UMC Utrecht, protocol number 11-320/K).

Recruitment

In a first screening (see Figure 1), roughly 17,000 families will be targeted through community records via two Dutch regional health care organizations (estimated response rate is 52%, see (Van Zeijl et al., 2007). All families will receive a personalized information letter, including the Eyberg Child Behavior Inventory (i.e., ECBI) to screen for children’s problem behavior (Eyberg & Robinson, 1983). The criterion for inclusion will be a score at or above the 75th percentile. This cut-off is chosen so that at-risk families will be selected, without excluding children and parents with subclinical or even normal-range functioning. Based on a conservative estimation, 889 families (10%) are expected to be eligible for inclusion. Additionally, a second screening will take place to check for exclusion criteria: mental retardation, IQ ≤ 70, not mastering the Dutch language, and or a val allele.

Figure 1. Study design (CONSORT schedule).
These families will receive a second invitation letter and will be contacted for trial participation.

Randomization

Participants will be informed of the design of the study and will give consent prior to randomization. Randomization will occur through random selection of a participant number that is linked to either the intervention or control condition.

Sample size calculation

Power analyses are essential to maximize chances to find significant G × E interactions. For this calculation, we used a Fixed Effects ANOVA Power Analysis in PASS11 (Hintze, 2011). Based on a meta-analysis that demonstrated a small effect size (d) of 0.20 of the IY intervention (Menting et al., 2013), and assuming a small G × E interaction effect and no main effects of genes (Caspi et al., 2002; Rutter et al., 1997, 2006), a sample size of N = 480 families will be required for investigating our hypotheses in a two-sided test at α = 0.05 and power (1-β) = 0.8.

Intervention

The IY training is aimed at improving parenting skills in order to reduce child behavioral problems, such as aggressive behavior, and enhance competent behavior. The IY training includes 14 to 16 weekly two-hour sessions. During these sessions parents watch video-vignettes, discuss parenting with each other and practice new techniques in role-plays. Each group will consist of approximately 10 to 12 parents. IY parent training is different from most other parent (management) training programs, in that trainers use a collaborative leading style: They do not instruct, but are part of the group and lead discussions (Webster-Stratton & Hammond, 1997; Webster-Stratton, 1994). Many previous randomized trials have shown the program to be effective (e.g., Hartman, Stage, & Webster-Stratton, 2003), also in The Netherlands (Posthumus, Raaijmakers, Maassen, Van Engeland, & Matthys, 2012). Hence, IY parent training is an evidence-based parent training.

Data collection

An overview of all measurement occasions is given in Table 1. Both the recruitment and, as well as the waves of data collection will be conducted in two separate cohorts in 2012/2013 and 2013/2014. The recruitment (i.e., screening) will take place in September–October 2012 and 2013. The pretests will take place in November-January 2012 and 2013. Families will be asked to fill out questionnaires in the presence of a researcher or research assistant during home visits. Furthermore, parent–child interactions will be videotaped during structured play situations. This procedure will be repeated twice after the pretest, namely at posttest and follow-up. Saliva samples for genotyping will be collected once during pretest.

Outcomes

Primary outcomes are the possible moderating effects of child genotype on the IY intervention effect (i.e., on the decrease in externalizing problems behavior and/or increase in prosocial behavior of the child). The intervention effect will be assessed with the ECBI, the Matson Evaluation of Social Skills with Youngsters (MESSY; Matson, Rotatori, & Helsel, 1983), and the Dyadic Parent–Child Interaction Coding System-Revised (DPICS-R; Eyberg, Nelson, Duke, & Boggs, 2005, Robinson & Eyberg, 1981), see measures in Table 1. Secondary outcomes will be the observed (changes in) emotional synchronization in parent–child interactions as possible underlying behavioral mechanism to the G × (G) × E interactions.

Analyses

First, using independent t tests, we will examine whether randomization was successful, comparing baseline levels of externalizing and prosocial behavior across the intervention and control condition. Possible significant differences at baseline will be used as covariates in analyses (Tabachnik & Fidell,
2007). Second, as the longitudinal data of individuals will be nested in families, multilevel latent growth curve analyses in Mplus (Muthén & Muthén, 1998-2010) will be performed. In this analysis, we will test the interaction between the variables ‘group’ (i.e., intervention vs. control) and ‘genotype’ (i.e., susceptible genotype vs. non-susceptible genotype) to examine possible G × E interactions. In addition, we will take into account families’ ethnic background (i.e., Caucasian vs. non-Caucasian) and additional parental support or (mental) health care families received during the study (i.e., additional self-sought care vs. no additional self-sought care). Ethnic background and additional care will both be used as covariates in the analyses.

**DISCUSSION**

The ORCHIDS study described in this protocol is a genetically informed randomized controlled trial targeting families with children aged 4-8 who show mild to (sub)clinical behavioral problems. The primary aim of ORCHIDS is to assess possible G × (G ×) E interactions in the development of child behavior in its full scope—that is, from children’s problem behavior to their competencies. Our large scale randomized controlled trial is one of the first experimental studies of G × E interactions in social development. Experimental manipulation of the environment is crucial in understanding G × E interactions, because it is the only way to prevent confounding gene-environment covariation.

Additional to a single gene approach, we will investigate possible cumulative effects of multiple candidate genes (i.e., G × G × E or polygenetic interactions) (e.g., Belsky & Beaver, 2011). Also, we will make a first attempt to obtain more insight into the behavioral mechanism underlying these G × E interactions by examining (changes in) emotional synchronization in observed parent-child interactions. Better insight into individual differences in, for example, reactivity to positive parenting behavior like praise, associated with dopaminergic and serotonergic functioning, may help improve the tailoring of behavioral parent training. This seems necessary as the mean effect size of these interventions is modest (Cohen’s d = 0.47; McCart et al., 2006). Individualization of use of rewards and praise may help increase the efficiency of these parenting skills. For example, if the emotional significance of the positive message of praise is less well processed, associated with altered dopaminergic functioning, both verbal and nonverbal enthusiasm may be particularly relevant for this specific subgroup of children (Matthys et al., 2012). Thus, instead of delivering interventions in a standardized way, parenting programs may benefit from an individualized approach based on insights from results of studies like the present one.

Despite the strengths and innovative aspects of ORCHIDS, there are some issues that our study is unable to take into account. Differential susceptibility to parenting may also be caused by environmental influences that alter the effects of genes (i.e., epigenetics), rather than by specific DNA sequences or a certain number of repeats alone (Szyf, Weaver, & Meaney, 2007). Human development is an active process powered by a continuous interaction between the genome and the environment (Meaney, 2010). DNA methylation (i.e., the biochemical process that involves the addition of a methyl group onto cytosine in the DNA, regulating the operation of the human genome), for example, has been shown to mediate the relation between genotype and developmental outcomes (Beach, Brody, Todorov, Gunter, & Philibert, 2010; Van IJzendoorn et al., 2011). Once differential susceptibility to the environmental manipulations has been demonstrated, a next step will be to further investigate the behavioral as well as neurobiological underlying mechanisms of genetic differences in sensitivity to change. Interpreting the intervention effect in this study will be like looking at an “omnibus effect” that covers a variety of possible environmental effects or change mechanisms; the environmental change induced by the intervention consists of changes in many different parent behaviors and child responses. Which of these changes are driving the omnibus effect cannot be elucidated in a randomized controlled trial. In order to create a more complete picture of gene-environment interplay, multiple genetically informed experimental designs should be used additionally to large scale randomized controlled trials such as micro trials: small-scale, randomized experiments using a brief and focused environmental manipulation, designed to suppress specific risk mechanisms or enhance specific protective mechanisms but not to bring about full treatment or prevention effects in outcome (Howe, Beach, & Brody, 2010).

**Conclusion**

The ORCHIDS study will investigate possible G × (G ×) E interactions in the development of both positive and negative child behavior by assessing whether an experimental manipulation of the environment with the Incredible Years intervention is more effective for a particular genetic subgroup of children than for others. With this study we will contribute to a further understanding of moderating effects of specific alleles (i.e., polymorphisms) on the malleability of child behavior, and the behavioral mechanisms that may underlie G × E. By doing so we gain more insight into what works for whom and how it works when it comes to interventions targeting child problem behavior.

**Trial Status**

The trial is ongoing, still recruiting participants.
Gene by Environment Research to Prevent Externalizing Problem Behavior: Ethical Questions Raised for a Public Healthcare Perspective
CHAPTER 4

GENE BY ENVIRONMENT RESEARCH TO PREVENT EXTERNALIZING PROBLEM BEHAVIOR: ETHICAL QUESTIONS RAISED FROM A PUBLIC HEALTHCARE PERSPECTIVE

KEYWORDS
- Gene by environment interactions
- Intervention
- Randomized-controlled-trial

PRESS

ABSTRACT
- The main public health advantages of examining gene by environment interactions (i.e., G × E) in externalizing behavior lie in the realm of personalized interventions. Nevertheless, the incorporation of genetic data in randomized controlled trials is fraught with difficulties and raises ethical questions.

This paper has been written from the perspective of developmental psychologists who, as researchers, see themselves confronted with important and in part new kinds of ethical questions arising from G × E research in social sciences. The aim is to explicate and discuss ethical questions, based on the conviction that what is ethically salient in a research setting will also be relevant in that area of public healthcare incorporating research findings. The ethical questions discussed include: whether it is ethically responsible to withhold an effective treatment; to what extent genetic results should be disclosed; whether researchers should be allowed to collect genetic data of both child and parent; and what are costs and benefits of personalized interventions based on (genetic) screening. We made an attempt to address these questions, but it is up to researchers to determine whether the solutions are suitable for their G × E research in social sciences.
Reliably identified and replicated $G \times E$ can thus help boost effectiveness of current interventions aimed at reducing externalizing problem behavior. In the long run, $G \times E$ research may even help to tailor such interventions to the needs of children and parents with specific constellations of genetic and family risk factors. Specifically, leading scholars suggested that in the long run, dependent on sufficiently replicable, generalizable, and explicable $G \times E$ findings, it might become possible to target various genetic subgroups with interventions differing in intensity, duration, and even clinical focus (Rutter, 2012). In this light, findings from recent experimental studies that provided evidence for differential responsivity to interventions based on child genetic-makeup are highly intriguing (for meta-analysis see Van IJzendoorn & Bakermans-Kranenburg, 2015). Nevertheless, $G \times E$ research—and the incorporation of genetic data in randomized controlled trials (RCTs) in particular—is fraught with difficulties and raises several serious ethical questions. This paper has been written from the perspective of developmental psychologists involved in conducting this kind of research and who, as researchers, see themselves confronted with important and in part new kinds of ethical questions arising from $G \times E$ research in social sciences. The aim is to explicate and discuss these ethical aspects, based on the conviction that what is ethically salient in a research setting will also be relevant in the area of public healthcare that is supposed to incorporate such like findings. First, we will explain the central concepts that are currently used in $G \times E$ research. Second, we will discuss ethical questions that researchers might encounter when conducting $G \times E$ research in a randomized controlled trial, which we call from now on a $G \times E$ trial. Partly, this will be based on our own experiences with conducting such a $G \times E$ trial involving genetic susceptibility to parenting behavior and the provision of a modified environment, in our case a parent management training. The ethical questions we encountered refer to: (i) withholding effective intervention, (ii) disclosure of genotyping results, (iii) collecting genetic data of both child and parent, and (iv) implementing $G \times E$ in clinical practice (see also Horstkötter et al., 2014; Horstkötter & De Wert, 2013; Munthe & Radovic, 2015 [this issue]; Singh & Rose, 2009).

$G \times E$ interactions

The expression ‘gene by environment interaction’ ($G \times E$) refers to the assumption that in order to understand human behavior neither genetic nor environmental factors should be evaluated in isolation, since most behavioral outcomes are the result of complex interactions between a certain genetic-makeup and specific environmental conditions. In social sciences, $G$ is mostly operationalized in terms of one single genetic factor (candidate gene) that is related to an individual’s sensitivity to a specific environmental (E) condition. In the context of developmental psychology, $G \times E$ means, for example, that the behavior of children carrying a specific allele of one defined gene, may be more negatively affected by adverse environmental conditions compared with other children who do not carry this specific allele, yet nonetheless suffer from the same conditions. More specifically, research found that children with the low-activity allele of the MAOA gene, a gene that is involved in the degradation of dopamine, more often developed a conduct disorder when being maltreated, compared to maltreated children with the high-activity allele of this gene (for meta-analyse see Byrd & Manuck, 2014; Kim-Cohen et al., 2006). The low-activity allele of the MAOA gene can therefore be considered a ‘risk allele’, yet the risk will only become manifested when exposed to an environmental risk factor. Or in reasoning the other way around, some environmental risk factors have more impact in children carrying the low-activity allele than in children without such an allele.

Also important here are so-called ‘gene × gene × environment interactions’ ($G \times G \times E$). $G \times G \times E$ refers to two different processes. First, it refers to an effect of two or more genetic factors (i.e., at least two alleles) that are related to an individual’s sensitivity to environmental conditions. The assumption is that children with two or more risk alleles may be more strongly affected by negative environmental conditions (i.e., polygenetic effect) than children with none or only one of the risk alleles. For example, one study found that the more risk alleles children carried, the less self-regulation they manifested under unsupportive parenting conditions (Belsky & Beavers, 2011). Second, $G \times G \times E$ have also been taken to refer to situations in which both parent and child carry a risk allele. This might affect both the parent and the child more strongly to negative environmental conditions, conferring a negative ‘double whammy’ effect of behavioral outcomes (Jaffee, Moffitt, Caspi, & Taylor, 2003). That is, parents with a risk allele may be more vulnerable to stress and thus show more maladaptive parenting when under stress themselves, and as a consequence their children who are also carrying a risk allele may in turn be more strongly affected by this maladaptive parenting.

Gene-based differential susceptibility

There is increasing evidence that the genetic factors commonly identified as making children more vulnerable for environmental adversity, and hence constitute a risk for developing externalizing problem behaviors, may instead function as ‘plasticity alleles’. That is the same children who are genetically most vulnerable when exposed to adverse environmental conditions may also be most positively affected by enriched environmental conditions as for example with warm, positive, and sensitive parenting. This idea is captured by the so-called ‘differential susceptibility model’ (Belsky et al., 2007, Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005). This model holds that some children are genetically more likely to be affected by parenting in a ‘for better AND for worse’ manner. Children will suffer severely if ignored or maltreated, but will flourish spectacularly when receiving adequate care. To put it in an widely used metaphor, it may be that we have overlooked that some children are like ‘orchids’—they are susceptible children who are fragile and fickle but who are also capable of blooming spectacularly if given greenhouse care. Yet other children may be rather like ‘dandelions’: resilient children who are able to take root and survive almost anywhere and even find their way in adverse environments. In this context, the polymorphisms long regarded as risk alleles might therefore be better regarded as ‘plasticity alleles’ A recent review (Belsky et al., 2009) and several single studies have suggested that

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Indeed specific polymorphisms located in various genes, for example DRD2, DRD4, DAT1, MAOA, COMT and 5-HTTLPR, should be considered to function as susceptibility rather than vulnerability factors (Belsky & Pluess, 2009).

At the moment, the tenability of this genetic differential susceptibility model is still unclear. Importantly, many G × E studies previously conducted did not meet all necessary preconditions for establishing differential susceptibility (Overbeek et al., 2012). One major issue is that they almost exclusively focused on dysfunction and environmental adversity. In doing so, however, these studies failed to address the question of whether susceptible children might also manifest more competence or happiness in response to environmental enrichment than their resilient peers.

The ORCHIDS study

From an empirical perspective it is important to overcome this limitation of G × E research. But also from a public health perspective it is particularly important to gain more knowledge on this issue, because several ethical concerns that take place when conducting G × E research are also relevant for public healthcare that is supposed to incorporate such like findings. To test genetically based differential susceptibility, we conducted a genetically informed RCT: the ORCHIDS study (Observational Randomized Controlled Trial on Childhood Differential Susceptibility; see Chiangur, Weel, Overbeek, Matthys, & Orobio de Castro, 2012). In this paper we will discuss questions on the basis of this study. Specifically, we tested whether a positive change in the parenting environment, based on the provision of the so-called ‘Incredible Years’ (IY) intervention, would be more effective in decreasing problem behavior in children with a plasticity allele than in children without such a plasticity allele. This study design would allow us to test the G × E interaction, between a stimulating parenting environment and the specific genetic make-up of children such approached. Also, we aimed to test G × G × E within the child as well as between the child and parents. This was done to test whether children with two or more plasticity alleles would be more strongly affected by an induced positive environmental condition, compared with children with none or only one of the risk alleles.

In our study, we invoked the IY intervention as a positive environmental stimulus that is able to realize the enriching environment that susceptible children respond to particularly well. IY aims to improve parenting skills in order to reduce children’s externalizing problem behavior, and enhance children’s prosocial and competent behavior (Webster-Stratton & Hammond, 1997; Webster-Stratton, 1994). Previous RCTs have provided evidence that the program is effective in ameliorating parental behavior and via this route, is able to reduce externalizing problem behavior in children (for meta-analysis see Menting et al., 2013).

We considered the main advantage of using an RCT design to increase statistical power and thereby to indeed identify potential G × E. This superior power is based on the active manipulation of an environmental factor of interest, which in our case, was dichotomized (i.e., intervention with and without plasticity alleles vs. control group with and without plasticity alleles). However, the envisioned incorporation of genetic data in this RCT raised several ethical questions directly linked to the domain of public healthcare. In the second part of this paper, we would now like to present and discuss these questions, because we think the ethical questions of G × E research regarding externalizing behavior have been given insufficient attention so far, while much emphasis is being put on potential clinical applications (Bakermans-Kranenburg & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015).

Ethical questions raised in G × E research

Withholding effective interventions

Like most healthcare RCTs involving human participants, G × E trials raise the question whether it is justifiable to withhold interventions from participants in order to accomplish scientific objectives. The debate contributes to the overall view that, although researchers usually do not have professional obligations to provide medical care to participants, they have ethical obligations to avoid exploiting them (Resnik, 2008).

In order to justify withholding (effective) treatment to the control group, researchers should clearly state the scientific objectives and clinical as well as potential social or public health gain of the study (Miller & Brody, 2002). In biomedical sciences, placebo-controlled RCTs are widely used as a first step of bringing new drugs into the market and justification of withholding treatment through scientific objectives seems to be very well accepted (Kokke, Bukstein, & Barron, 1999, Punja, Zorzel, Hartling, Urichuk, & Vohra, 2013, Wolraich et al., 2001). By contrast, the incorporation of biological or genetic data, and the use of control groups, currently seems to be less accepted in social sciences. Research in this area most often solely uses psychological measurement data rather than participants’ genetic data and uses waiting list conditions rather than control conditions. However, G × E trials incorporate both by including psychological measurements and genetic data to gain knowledge in both psychological as well as in biological processes. Thus, a traditional division between biological and social behavioral disciplines seems no longer justifiable: G × E research transgresses previous boundaries and contains a biological and a psychological compound that are closely related to individual outcomes. Initially this has been met with reluctance in the social sciences to accept G × E trials in which control groups are withheld; certain interventions may well be reconsidered. We argue that withholding an effective intervention to control groups in a G × E trial setting in social sciences can be justified, on the ground that it is an empirical necessity and provided that these individuals are permitted to receive care as usual and are therefore not withheld of (mental) healthcare, as was the case in the ORCHIDS study.

The scientific objective of the ORCHIDS study was to test the differential susceptibility...
hypothesis. More specifically, we wanted to examine whether children with (more) plasticity alleles—who have been previously shown to do worse under negative parenting conditions—will indeed do better under positive parenting conditions compared to their resilient peers without those plasticity alleles. By testing whether these genetically based susceptible children showed most improvement after their parents received the IY intervention, we tested the ‘for better’ part of the differential susceptibility equation. In doing so, we hoped to gain insight into the malleability of these susceptible children who, for a long time, had been thought to be not susceptible, but vulnerable and fragile. But to get to know whether any behavioral change in these children’s behavior is due to an enriching environment (instead of for example children simply aging), we needed to be able to compare children who received intervention to a control group.

In the ORCHIDS study we used the evidence-based IY intervention, which was only offered to parents in the experimental group. The effectiveness of the training has been shown multiple times (for meta-analysis see Menting et al., 2013). This might lead to the thought that a control group in a RCT is apparently withheld from an effective intervention. However, a control group in this case is an empirical necessity to generate evidence that participants are indeed being differentially susceptible to preventive interventions. Such evidence might create more realistic expectations of intervention efficacy. Weak intervention effects might lead to a dead end, not only in G × E research but also in terms of policymaking in public healthcare. Policymakers might, in turn, be concerned about limited interventional impacts and cost-effectiveness and thus they might be less inclined to support interventions or to roll out evidence-based interventions on a larger scale when its effectiveness is modest. This way the intervention effect might in fact be underestimated for a specific group: the susceptible children with a plasticity allele (and possibly overestimated for children without such a plasticity allele). G × E trials, with a control group, can generate a basis of proof for this relatively new idea (Bakermans-Kranenburg & Van IJzendoorn, 2015).

One might also argue that if we take the assumption of differential susceptibility seriously, we should take into account the possibility that an intervention may be offered to (sub)groups of families—that is the resilient ones—for whom this intervention might not be that effective. At the same time this means that the control group, who do not receive intervention, will contain similar families who anyhow would not have profited very much from the intervention and for whom it might make little sense to argue that they are withheld from an effective intervention. However, it does not follow that those who prove relatively resilient (i.e., less susceptible) do not benefit at all from any interventions. Notably, it just might be that the less susceptible children need an expanded duration, range, or intensity to reach the same effect as the more susceptible ones. Thus, withholding an effective intervention would do harm to these families (Belsky & Van IJzendoorn, 2015). More research is needed to test whether this is truly the case, but until then, families with children carrying none or fewer plasticity alleles should not be excluded from any intervention solely based on their genetic makeup.

Taken together, in a G × E design it is unclear what should count as withholding of treatment. But as soon as the possibility of plasticity alleles is taken into account and we want to gain more knowledge on differential susceptibility to intervention, a situation of equipoise might come into existence. This might justify the set-up of G × E trials that makes use of a control group in order to investigate—again—an intervention that otherwise had been recognized as a small to moderate effective program. Only this design allows us to gain insight into potential differences in susceptibility, to improve our ability to determine what works for whom, and to avoid old-fashioned ‘one size fits all’ approaches in the future (Belsky & Van IJzendoorn, 2015).

Disclosure of results

The rapid expansion of knowledge on human molecular genetics has led to an extensive debate about whether genetic data should be disclosed to participants (Jarvik et al., 2014; Quaid, Jessup, & Meslin, 2004; Savulescu & Skene, 2012). Main arguments for full disclosure whatsoever are that participants expect an element of reciprocity when participating in research (Hoefer, 2010), that disclosure may be the main motivation to participate (e.g., Sutrop & Simm, 2004), and that participants should be informed about any results that may be valuable to their (psychological) well-being (Knoppers, Joly, Simard, & Durocher, 2006). Main arguments for not disclosing results are that participants are not capable of adequately interpreting genetic information, leading to unclear or false conclusions (Klitzman, 2006) and that social scientists do not have the appropriate expertise to communicate results on genetics at a clinical level (Clayton & Ross, 2006). Although no consensus on this issue has yet been reached, the extreme positions of either full disclosure or no disclosure whatsoever have seldom been defended. On a middle ground, therefore, scholars stated that further discussion should no longer address whether (genetic) data should be disclosed, but instead should address how best to make an appropriate selection of results to be disclosed (see Bredenoord, Kroes, Cuppen, Parker, & Van Deiden, 2011; Bredenoord, Onland-Moret, & Van Deiden, 2011).

In line with Bredenoord, Onland-Moret, and colleagues (2011), we believe that the ethical question that should be addressed is: Under which circumstances should results be disclosed to participants? However, there are a few things we need to take into account before discussing this further. First of all, research on G × E interactions is still in its infancy and findings need replication and extension before we can know their full implications (e.g., Duncan & Keller, 2011; Rutter, 2012). Second, to date G × E results are only applicable at a group level. Most processes in developmental psychology are non-ergodic, meaning that results at the group level do not automatically hold true for each individual within that group (Molenaar, 2008). Third, there is a problem with explaining genetic results to children. Research in G × E in social
science, and particular in developmental psychology, often includes under-aged children. In accordance, parents need to give their informed consent for the use of their children’s genetic data. Full disclosure would mean that parents are responsible for explaining such information—on whether their child has certain specific alleles or not, and what this means precisely—to their child, because the results are primarily disclosed to the parents (Hamilton, Bowers, & Williams, 2005). This may lead to an ineffective or incorrect communication of the results to children, which would be of little use. Another problem is that this information has no individual clinical value. Moreover, even if it would have individual clinical value, children may not want to know this information—and in this procedure it would be hard for researchers, who are responsible for an adequate and ethically sound dissemination of their research findings, to monitor this process (Tarini, Tercyak, & Wilfond, 2011).

In the ORCHIDS study we decided that informing parents about their individual child’s genetic information would offer no immediate personal benefit but instead might give families an unnecessary ‘overload’ of information that is difficult to interpret. Also, such information may give rise to false genetic determinism in parents and may create adverse developmental effects in families. For example, based on information about their child’s genotype, parents might believe their child is at increased risk for adversity and pathological development, and because of this they may treat their child differently (e.g., increasing their strict behavioral control or even administering harsh discipline). Nevertheless, we hypothesized that disclosure on a group level would do no harm. In accordance, we decided to give parents the results of the study on G × E for the total sample, to which they belonged. Thus, as parents may expect an element of reciprocity and may be more motivated to participate when outcomes are communicated, we decided to do so in an accessible and nuanced way without providing them information about the genetic make-up of their individual child (e.g., popular scientific article and newsletters, see Knoppers et al., 2006). We realize that disclosure of genetic data might be questioned not only in a research setting, but particularly also in a related—future—clinical setting. Critical scholars, for example, uttered the concern that resilient children might be increasingly ignored or left helpless (Wasserman, 2004). We will revert to this point in due course.

Collecting genetic data from children and parents

The next step for G × E trials might be to investigate the genetic effect of multiple plasticity alleles in combination with an environmental condition (i.e., G × G × E) rather than with one plasticity allele (i.e., G × E). The possession of a plasticity allele in parent and child might confer a ‘double whammy’ effect (Jaffee et al., 2003): extra heightened susceptibility, due to possessing more than one plasticity allele. In the ORCHIDS study we wanted to investigate this effect. To that end we hypothesized that parents with a plasticity allele would benefit the most from the IY intervention and that, in turn, their significant change in positive parenting behavior would be cumulatively beneficial if their child also carried this plasticity allele. To date, however, such G × G × E effects have gone almost entirely untested in genetically informed research designs. One reason for this seems to be that G × G × E research generates or is faced with new research-related ethical dilemmas.

A further ethical question might consist in the additional burden of parents when their genetic sample is collected. One might argue that this burden, even though very small (i.e., one saliva swab), is unnecessary since the scientific yield may be minimal. In this case, scientific yield may be minimal because the strong genetic association between parent and child (i.e., heritability) might lead to an underpowered test of effect (i.e., there are only a few families in which children and their parents are not genetically alike). Indeed there is a great overlap of about 50 per cent between parents’ DNA and that of their offspring. In our view, however, this line of reasoning does not withstand close scrutiny. Given that every gene has two alleles, from which the child inherits one of the mother and one of the father, the actual genetic overlap between a given parent and offspring can be much smaller than 50 per cent. That, however, might require the separate collection of parental genetic data, or at least it would require an explicit discussion of how much, or how little, overlap is needed to consider the separate collection of parental DNA either justified or not. This discussion, which could be considered a discussion on the specific risks and benefits of an innovative research approach, will have to take place with local IRBs in charge of approving such projects. Likewise, however, we would like to argue it should take place in the bioethical discourse that from a theoretical point of view will have to settle the ethical boundaries of research beyond former disciplinary boundaries.

Implementation of knowledge on G × E in clinical practice

The aim of G × E research in public healthcare is to gain knowledge on what works for whom, in order to tailor interventions in duration, intensity and clinical focus. Even though public healthcare might benefit from such kind of research in the long run, its application might also raise several ethical questions. Specifically, it raises the question of how to implement the knowledge that some children, the resilient ones, are less or non-responsive to interventions in an ethically justifiable way. The danger lurks that these children will not be prioritized to receive the intervention although they need help as much or possibly even more than others. These children, however, may well benefit from interventions that are tailored to their specific neurobiological characteristics (Matthys et al., 2012). Thus, especially for these potentially less susceptible children it seems important to develop a rich array of care and assistance and thus this group should never be neglected socially because of their genetically predisposed resilience (Ellis, Boyce, Belsky, Bakermans-Kraenenburg, & Van IJzendoorn, 2011).

Another ethical question that comes up is: How valid the implications of G × E trials then really are for public healthcare? And even if we get to the point of reliable implications—how are we going to identify the more plastic families that are supposed to be most (or even exclusively) susceptible for an intervention? Are we going to apply
genetic testing? Some scholars predict that genetic testing will become increasingly important as a guide to prevention, drug treatment, and clinical management (Burke et al., 2002; Van Gooren & Fairchild, 2008). But as Munthe and Radovic (2015) [this issue] argued, even if we could locate an inherent plasticity characteristic through genetic screening, no evidence could be found that such a trait would manifest itself as a plasticity factor; this is because it is the interaction with the environmental condition itself that reveals such a factor. Therefore, we also need knowledge on ‘why’ these children are more susceptible. Specifically, we need to know which neurobiological endophenotypes underlie such an interaction between G and E. In addition, genetic screening could present collective risks to an identifiable subgroup (Sharp & Foster, 2000). For example, a genetic screening that associates a plasticity allele with a genetic disposition for externalizing behavior problems could lead to group stigmatizing and discrimination (Rodriguez, 2012; Viding, Larsson, & Jones, 2008). Thus, we are still far from a point where researchers can claim that we should give an intervention to some children and not to others due to their genetic make-up. With the current state of knowledge, genetic screening for differential susceptibility would lead to too many false decisions (Belsky & Van IJzendoorn, 2015). However, it might very well be that in the future we come to a point where we gain knowledge into neurobiological endophenotypes associated with genetic plasticity, which makes (genetic) screening in the context of preventive intervention more appropriate.

Conclusion

Similar to what is currently happening in modern (bio)medicine, it is important to gain insight into individual differences in ‘treatment responsivity’ to behavioral interventions. Several candidate genes have been proposed as markers for such differences in responsivity. Increasing our knowledge on G × E may help to tailor personalized interventions, and in turn boost the currently small to modest effectiveness of interventions aimed at reducing children’s externalizing problem behavior (Bakermans-Kranenburg & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015). However, there are several ethical questions involved in conducting G × E trials to shape such intervention strategies. We made an attempt to address some of these questions that social scientists in this field often encounter when designing a study. We have offered a description of ethical considerations in the ORCHIDS study and the chosen solution. It is up to researchers to determine whether these solutions might be suitable for their G × E trials. However, even if researchers are able to effectively resolve these questions, they should not neglect additional concerns about implementing G × E results in public healthcare and should prepare for ethically sound future practices. How this can be realized, however, needs further debate.

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Intervention Effectiveness of The Incredible Years: New Insights into Sociodemographic and Intervention-Based Moderators.
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**KEYWORDS**
- Prevention
- Randomized-controlled-trial
- Externalizing disorders
- Incredible Years
- Moderators

**PRESS**

**ABSTRACT**

We tested the effectiveness of the preventive behavioral parent training (BPT) Incredible Years (IY) and the effects of previously suggested sociodemographic and intervention-based moderator variables (i.e., initial severity of externalizing behavior, child gender, social economic status, family composition, and number of sessions parents attended), in a large scaled randomized-controlled-trial. Using full intention-to-treat analyses, questionnaire and observation data from 387 parents and children aged 4-8 years (Mage = 6.21, SD = 1.33; 55.30 % boys), across pretest, posttest, and 4-month follow-up, were analyzed, correcting for multiple testing. IY was successful in decreasing parent-reported child externalizing behavior (Cohen's d = .20 at posttest; d = .08 at follow-up), increasing parent-reported positive parent behavior (d = .49; d = .45) and observed behavior (d = .06; d = .02), and decreasing parent-reported negative parenting behavior (d = .29; d = .25). No intervention effects were found for reported and observed child prosocial behavior, observed child externalizing behavior, and observed negative parenting behavior. Out of 40 tested moderation effects (i.e., eight outcomes times five moderators), only three significant moderation effects appeared. Thus, no systematic evidence emerged for moderation of IY effects. The present multi-informant trial demonstrated that many previously suggested moderators may not be as potent in differentiating BPT effects as once thought.

Negative parenting behaviors and strategies, such as disapproval, inconsistent discipline, harshness, and psychological control have been related to externalizing behavior in children and adolescents (e.g., Bor, Sanders, & Markie-Dadds, 2002; Collins, Maccoby, Steinberg, & Hetherington, Bornstein, 2000; Ge, Brody, Conger, Simons, & Murray, 2002; Karremans et al., 2006; Rothbaum & Weisz, 1994). Negative parenting behaviors have also been found to mediate the relation between more distal family risk factors (e.g., socioeconomic status, parental psychopathology) and child externalizing behavior (Dodge, Coie, & Lynam, 2006; Reid, Patterson, & Snyder, 2002). In contrast, positive parenting behaviors and strategies, such as acceptance, appropriate discipline, responsiveness, and limit setting have been related to child prosocial behavior (Zahn-Waxler, Iannotti, Cummins, & Denham, 1990). The most effective interventions aimed at reducing externalizing and promoting prosocial child behavior have therefore been designed to target both negative and positive parenting behaviors (McCart et al., 2006). Specifically, behavioral parent training programs (BPT) use parents as agents by training them in using parenting strategies that create positive changes in parenting behavior and, through this, indirectly lead to positive changes in child behavior.

Effectiveness of BPT in reducing child externalizing behavior and promoting prosocial behavior has been proven in multiple independent studies, but effect sizes are moderate (McCart et al., 2006; Menting et al., 2013; Reyno & McGrath, 2006). One way to boost effectiveness is by taking into account factors that determine intervention effectiveness. However, our insights into such moderators is limited due to scarcity of studies and several specific methodological issues, such as small sample sizes, low statistical power, and assessment of effects that are limited to questionnaire information. Moreover, the direction of the suggested moderators is unclear and to date the moderators have been studied separately in different studies, or inferred from comparisons of effect sizes between studies. In order to control for possible confounding effects of the different moderators, assessment within a single RCT with sufficient statistical power to do so is required. This will be the scope of the present paper – building not only on parent-reports but also on observational data on parenting and child behaviors. Because we measured the degree of beneficial effects of Incredible Years in a “real world” prevention setting, working through health care institutions, this study can be seen as an effectiveness trial.

BPT is an effective method to reduce child externalizing behavior and promote child prosocial behavior in different populations (for meta-analyses see McCart et al., 2006; Menting et al., 2013; Sandler et al., 2011). However, the mean effect size of BPT in indicated prevention settings is relatively modest (d = 20), compared to the mean effect size in treatment settings (d = 50) (McCart et al., 2006; Menting et al., 2013; Reyno & McGrath, 2006). In addition, the effectiveness of BPT programs appears to be influenced by sociodemographic moderators (Gardner, Hutchings, Bywater, & Whitaker, 2010; Scott & O'Connor, 2012) and intervention-based moderators (Wilson & Lipsey, 2001). Recent meta-analyses on both the IY program (Menting et al., 2013),...
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as well as on BPT in both prevention and intervention settings more broadly defined (Leijten, Raaijmakers, Orobio de Castro, & Matthys, 2013; Lundahl, Risser, & Lovejoy, 2006), suggested that specifically initial severity of child externalizing behavioral problems, child gender, socioeconomic status (i.e., SES), family composition (i.e., single parent families vs. two-parent families), and the number of sessions parents attended might be important moderators of the intervention effects of the BPT program IY.

Initial severity of externalizing behavior

Initial severity of child externalizing behavior is one of the strongest predictors of intervention effects (Leijten et al., 2013; Lundahl et al., 2006; Menting et al., 2013). However, moderation has been found in both directions. On the one hand, initial severity might account for a threshold that confers advantage in terms of intervention effectiveness; larger initial severity leaves more room for improvement (Gardner et al., 2010). Also, larger initial severity might be related to increased motivation in parents to change, leading parents to more readily agree to and engage in treatment (i.e., larger treatment adherence). On the other hand, it has also been found that initial severity of externalizing behavior could reduce intervention responsivity (e.g., Kazdin, 1995, Runa, Burke, & Thompson, 1996). Possibly, this is because more severe levels of externalizing behavior are related to increased numbers of child, parental and environmental risk factors (e.g., comorbid psychopathology, severe child dysfunctioning, parental stress, and parents’ perception of failing), that in turn negatively affect parents’ motivation and engagement in BPT. Initial severity of externalizing behavior might therefore be a specifically important moderator of effectiveness in a prevention setting, where severity might vary more between families, compared to treatment settings.

Child gender

Child gender might be another moderator of BPT effectiveness. A previous study suggested that the effects of BPT in a prevention setting were stronger for boys than for girls (Gardner et al., 2010, but see review McMahon, Wells, & Kotler, 2008) for conflicting results). However, the influence of child gender as a moderator might partly be due to confounding effects of initial severity (i.e., boys showing more externalizing behavioral problems than girls) (Menting et al., 2013). It might therefore be specifically important to control for initial severity when testing for possible moderating effects of gender.

SES

Although the meta-analysis by Lundahl and colleagues (2006) suggested that BPT is less effective for economically disadvantaged families, a recent meta-analysis showed that—when initial externalizing behavior was controlled for—economically disadvantaged and advantaged families benefited equally from the intervention effects directly post intervention (Leijten et al., 2013). SES and initial problem severity are likely to be confounded. Therefore, the unique influence of SES is unknown.

Family composition

It has been suggested that single parents possibly benefit less from BPT programs compared to two-parent families, which may be linked to limited financial resources, fewer coping resources, and/or greater isolation in single parent families (e.g., Griffin, Batvin, Scheier, Diaz, & Miller, 2000). However, findings on single parenting as a moderator of BPT effectiveness in indicative prevention and treatment settings are inconclusive (e.g., Kazdin, 1995, Reyna & McGrath, 2006, but see Fassum, March, Handegård, Drugli, & Larsson, 2009; Gardner et al., 2010) for conflicting results). Single parents and/or parents from a low SES background might be less able to attend sessions due to a lack of social and economic resources to meet preconditions for attending, such as transportation and child care. Again, this moderator might therefore be confounded with other possible moderators (SES and number of sessions attended).

The number of intervention sessions parents attended

Meta-analyses by Wilson and Lipsey (2001) and Menting and colleagues (2013) suggested that a higher amount of sessions parents attend is positively related to effect sizes of BPT in both prevention and treatment settings. BPT programs teach specific parenting techniques and during training sessions parents have opportunities to see how such techniques can be implemented, practiced, and refined. Missing one or more training sessions means missing specific intervention content. The number of sessions parents attend might therefore be relevant for intervention success (i.e., dosage effect). However, the number of sessions attended might be associated with other moderators, specifically SES and family composition. Therefore, it is important to investigate possible confounding effects of different moderators.

Methodological limitations of previous studies

Besides conflicting findings about the direction and unique (vs. confounding) effects of moderators of intervention effectiveness, the reviewed findings should be viewed as preliminary because of several methodological limitations that have plagued previous studies (see Weersing & Weisz, 2002). Specifically, most previous research on preventive BPT programs relied exclusively on parent reports of both parenting and child behavior. However, these reports might be biased and confounded (Sessa, Avenevoli, Steinberg, & Morris, 2001; Stifter, Willoughby, & Towe–Goodman, 2008). By providing a blinded assessment of changes in parenting and child behavior, observations besides questionnaires have important methodological advantages, (Daley et al., 2014, Scott, 2001; Sonuga-Barke et al., 2013). In addition, most intervention studies have a modest sample size. For instance, studies incorporated in the meta-analyses
on IY intervention effects by Menting and colleagues (2013) contained on average 95 families. This is problematic given that small sample sizes lead to an increased risk for both Type I error (i.e., incorrectly concluding there is an effect) and Type II error (i.e., concluding there is no effect when one actually exists). This is especially the case in moderation analyses where the sample is split up in multiple subgroups (Sullivan & Feinn, 2012). Therefore, a rigorous evaluation trial of preventive BPT is required, including observational assessments of both parenting and child behavior, with a sufficiently large sample size to test the effects of specific sociodemographic and intervention-based moderators. The current study tested moderation (i.e., initial severity of externalizing behavior, child gender, SES, family composition, and number of sessions parents attended) of the effectiveness of the BPT program IY in an indicated preventive context, by assessing parent reports and observational data on both child and parenting behavior, within one multivariate model, controlling for the possible confounding effects of the moderators. This study can mainly be seen as an effectiveness trial as it was conducted in conditions of routine clinical practice.

**METHODS**

**Design**

The current study is a randomized controlled indicated prevention trial with two conditions (intervention vs. control) and three measurement waves (pretest, posttest, and follow-up). It was built up in two stages. In stage one, all families with children aged 4-8 in the targeted municipalities were invited for a screening. In stage two, all eligible families were invited to participate in an RCT: the Observational Randomized Trial on Childhood Differential Susceptibility (i.e., The ORCHIDOS study). Enrolled families participated in the following three waves: pretest before randomization, posttest immediately after the intervention (i.e., 4 months after pretest procedure), follow-up 4 months after intervention (i.e., 8 months after pretest procedure). Randomization to either control or experimental condition (1:1) occurred after pretest and consent to participate. An independent researcher drew a ticket (which read either control or experimental condition) that was put back afterwards. Both assessors and parents were blind to allocation status at initial assessment.

**Screening**

Families were screened and recruited through community records via two Dutch regional health care organizations. All families with children aged 4-8 years (N = 20,048) of four (i.e., two large and two small) municipalities received a personalized information letter, including a consent form and the screening questionnaire (i.e., Eyberg Child Behavior Inventory; Eyberg & Pincus, 1999). Families were offered €7.50 for returning the questionnaire within two weeks. A total of 5,876 questionnaires were returned timely (response rate 22.52%). Children scoring at or above the 75th percentile of their relative cohort (i.e., sum score of 112 for girls and 120 for boys aged 4 and 5, 107 for girls and 116 for boys aged 6 to 8, 110 for girls and 115 for boys aged 4 and 5, 106 for girls and 112 for boys aged 6 to 8, for the two cohorts respectively) were eligible for participation in the study (N = 1,524). One parent-child dyad per family (N = 1,393) was invited to participate. Parents of either sex and of any ethnic group (mastering the Dutch language) were eligible. Eligible families received an invitation letter to participate in the RCT. One week later parents were individually contacted by a researcher or trained research assistant who briefly explained the study process. We were able to reach approximately 61% (N = 850) of eligible families, of which 46% agreed to participate (see Figure 1 for an overview on the selection-process of participants). The ECBI intensity scores of participating and non-participating children slightly differed (F(1, 1,40) = 6.66, p = .01), in that parents’ perceptions of children’s externalizing behavior were higher in participating families (M = 3.65; SD = .45) than in families who did not participate (M = 3.58; SD = .46).

**Participants**

In total, 387 parent-child dyads eventually participated in the RCT. Children were between 4 and 8 years of age at baseline (Mage = 6.31, SD = 1.33), mostly born in The Netherlands (97.4%), and about half of them (55.30%) were boys. Participating parents (91% mothers) were between 23 and 51 years of age at baseline (Mage = 38.10, SD = 4.84), mostly born in The Netherlands (i.e., 86% of mothers, 84% of fathers), and about half of them completed a higher form of education (i.e., higher vocational training or university level educational tracks) (see Tables 1-3 for demographic and descriptive statistics). For descriptive purposes of our sample, parents also reported—on a 3-point scale (1 = not true to 3 = certainly not true)—on levels of child peer problems (M = 1.16; SD = .42), hyperactivity (M = 1.34; SD = .45) than at pretest (i.e., Strength and Difficulties Questionnaire, Goodman, 1997). About a third (28.6%) of participating families received additional (mental health or family) care or help (e.g., mental health care parents or social services) and 8% of children used psychoactive medication (mainly psychostimulants) between pretest and posttest.

**Randomization check**

Participants in the intervention and control condition did not significantly differ in age (child or parent), gender (child or parent), country of birth (child or parent), parental education level, work status, marital status, religion, parent-reported and observed parenting behavior, and parent-reported child behavior at baseline (ps > .06) (see Tables 1-3 for descriptive statistics). Observed negative child behavior significantly differed between the two conditions (F(1, 1,61) = 5.40, p = .02), indicating that children in the intervention condition scored higher on observed negative behavior (M = 52; SD = 62) compared to children in the control condition (M = 39; SD = .46). This difference was controlled for in all analyses.
During the study 28 families dropped out, of which 23 at posttest and 5 at follow-up. Reasons for dropping out were inability to reach parents, (upcoming) divorce of parents, and/or moving house. There was no difference between conditions in the number of families that dropped out of the study (χ² (8, N = 386) = 21.52, p < .01). Mothers who participated in all three waves were more timely, p < .05. Reasons for dropping out were inability to reach parents, (upcoming) divorce of parents, and/or moving house. There was no difference between conditions in the number of families that dropped out of the study (χ² (8, N = 386) = 21.52, p < .01). Mothers who participated in all three waves were more timely, p < .05.

Dropouts

During the study 28 families dropped out, of which 23 at posttest and 5 at follow-up. Reasons for dropping out were inability to reach parents, (upcoming) divorce of parents, and/or moving house. There was no difference between conditions in the number of families that dropped out of the study (p < .19). When comparing parents that participated in all three waves with parents who dropped out, no significant differences were found at baseline regarding reported and observed measurements (p > .09). Also, we found no significant differences regarding sociodemographic and intervention variables except for marital status (χ² (4, N = 386) = 11.30, p = .02) and mother’s education level (χ² (8, N = 386) = 21.52, p < .01). Mothers who participated in all three waves were more likely to be married (71% vs. 51% married) and higher educated (81% vs. 51% high educated), compared to parents who dropped out during the study.

Table 1. Sample Demographics

<table>
<thead>
<tr>
<th>Age child (in years)</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (N = 387)</td>
<td>C (n = 190)</td>
</tr>
<tr>
<td>6.51</td>
<td>1.33</td>
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<table>
<thead>
<tr>
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<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
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</thead>
<tbody>
<tr>
<td>Boy</td>
<td>55.10</td>
<td>57.00</td>
<td>53.60</td>
<td></td>
</tr>
<tr>
<td>Girl</td>
<td>44.70</td>
<td>42.00</td>
<td>47.40</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Country of birth mother</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
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</thead>
<tbody>
<tr>
<td>The Netherlands</td>
<td>86.00</td>
<td>84.80</td>
<td>87.40</td>
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<tr>
<td>Other</td>
<td>14.00</td>
<td>15.20</td>
<td>12.60</td>
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<table>
<thead>
<tr>
<th>Country of birth father</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
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<tbody>
<tr>
<td>The Netherlands</td>
<td>84.50</td>
<td>83.80</td>
<td>85.80</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>15.50</td>
<td>16.20</td>
<td>14.20</td>
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<table>
<thead>
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<th>Age parent (in years)</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
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</thead>
<tbody>
<tr>
<td>58.15</td>
<td>48.48</td>
<td>58.02</td>
<td>49.00</td>
<td>58.17</td>
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<table>
<thead>
<tr>
<th>Gender parent</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>8.00</td>
<td>7.60</td>
<td>8.40</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>91.00</td>
<td>92.40</td>
<td>91.60</td>
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<table>
<thead>
<tr>
<th>Education mother</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
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<tbody>
<tr>
<td>Low</td>
<td>21.20</td>
<td>23.50</td>
<td>18.90</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>27.50</td>
<td>27.00</td>
<td>27.10</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>50.50</td>
<td>48.00</td>
<td>52.60</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Religion</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christian</td>
<td>59.15</td>
<td>40.30</td>
<td>56.85</td>
<td></td>
</tr>
<tr>
<td>Islamic</td>
<td>5.45</td>
<td>5.10</td>
<td>5.75</td>
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</table>

<table>
<thead>
<tr>
<th>Marital status parent</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married/ living together</td>
<td>87.00</td>
<td>85.70</td>
<td>88.40</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>8.80</td>
<td>11.20</td>
<td>9.50</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4.10</td>
<td>3.10</td>
<td>5.50</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Children in family (HLCC)</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.25</td>
<td>0.82</td>
<td>2.50</td>
<td>0.87</td>
<td>2.24</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Employment parent</th>
<th>Total (N = 387)</th>
<th>C (n = 190)</th>
<th>C (n = 197)</th>
<th>C (n = 199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employed</td>
<td>75.60</td>
<td>75.60</td>
<td>71.60</td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>26.40</td>
<td>24.40</td>
<td>28.40</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>23.50</td>
<td>19.60</td>
<td>26.40</td>
<td></td>
</tr>
<tr>
<td>Stay at home parent</td>
<td>44.40</td>
<td>45.50</td>
<td>45.30</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>52.70</td>
<td>56.90</td>
<td>28.10</td>
<td></td>
</tr>
</tbody>
</table>

Note. *descriptive statistics participating parent only. low = completed middle or high school; medium = completed vocational training; high = completed higher vocational training or university level educational tracks.

Procedure

At each measurement wave, parent-child interactions were filmed during a structured play situation and parents filled out a digital questionnaire. During pretest, researchers or trained research assistants took time to explain the study in more detail, to answer questions, and in turn asked parents to sign the informed consent form. During this wave, pretest questionnaire data were collected, parent-child interactions were filmed during a structured play situation, and saliva samples for genotyping were collected (Chhangur, Weeland et al., 2012). During posttest and follow-up assessment, the observation and questionnaire procedures were repeated. In addition, parents were interviewed by a trained researcher about children’s genetic ancestry (i.e., country of birth great-grandparents) and family mental health care
negative parenting behavior, we combined the scales harsh and inconsistent discipline and physical punishment. Reliability for both scales was satisfactory on all measurements (positive parenting behavior $\alpha > .70$, negative parenting behavior $\alpha > .78$).

Eyberg Child Behavior Inventory (ECBI)

The ECBI assesses the occurrence of conduct problems in children aged 2 to 16 years (Eyberg & Pincus, 1999). We used the ECBI intensity scale consisting of 36 items, which measures the frequency of the problem behavior (e.g., ‘Acts defiant when told to do something’) on a 7-point scale (1 = never to 7 = always). Reliability of the intensity scale was good for all three measurements ($\alpha > .84$).

The Matson Evaluation of Social Skills with Youngsters (MESSY)

The MESSY assesses social skills in school-aged children (Matson et al., 1983). The questionnaire consists of 62 items measured on a 5-point Likert scale (1 = not at all to 5 = very much) assessing the frequency of 2 types (i.e., prosocial and aggressive) of behavior in a range of social situations. In the current study, the scale appropriate social behavior was selected to measure prosocial behavior. The scale consists of 20 items (e.g., ‘Sticks up for friends’) and reliability was good for all three measurements ($\alpha > .88$).

Observational measure

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 pretest, posttest, and follow-up. The observation procedure

Table 2. Descriptive Statistics of Child and Parent Behavior Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pretest Mean</th>
<th>Pretest SD</th>
<th>Posttest Mean</th>
<th>Posttest SD</th>
<th>Follow-up Mean</th>
<th>Follow-up SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child behavior measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed positive behavior</td>
<td>2.38</td>
<td>.96</td>
<td>2.43</td>
<td>.95</td>
<td>2.32</td>
<td>.97</td>
</tr>
<tr>
<td>Reported positive behavior</td>
<td>4.65</td>
<td>.62</td>
<td>4.70</td>
<td>.63</td>
<td>4.67</td>
<td>.61</td>
</tr>
<tr>
<td>Observed negative behavior</td>
<td>1.56</td>
<td>1.06</td>
<td>1.54</td>
<td>1.05</td>
<td>1.58</td>
<td>1.07</td>
</tr>
<tr>
<td>Reported negative behavior</td>
<td>2.75</td>
<td>.61</td>
<td>2.80</td>
<td>.62</td>
<td>2.70</td>
<td>.59</td>
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<tr>
<td>Parent behavior measures</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed prosocial behavior</td>
<td>1.33</td>
<td>.64</td>
<td>1.34</td>
<td>.67</td>
<td>1.32</td>
<td>.61</td>
</tr>
<tr>
<td>Reported prosocial behavior</td>
<td>.64</td>
<td>.61</td>
<td>.64</td>
<td>.66</td>
<td>.64</td>
<td>.57</td>
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<tr>
<td>Parent behavior measures</td>
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<tr>
<td>Total Intervention Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Intervention Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 3. Correlations between Sociodemographic and Intervention-based Moderators

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Pretest</th>
<th>Posttest</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child gender</td>
<td>-.205**</td>
<td>-.208**</td>
<td>-.205**</td>
</tr>
<tr>
<td>Family composition</td>
<td>.105*</td>
<td>-.058</td>
<td>-.134**</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>.075</td>
<td>-.179**</td>
<td>-.163**</td>
</tr>
<tr>
<td>Number of previous placements</td>
<td>.039</td>
<td>.073</td>
<td>.087</td>
</tr>
</tbody>
</table>

Note. aEyberg Child Behavior Inventory; bSum scores; cDyadic Parent-Child Interaction Coding System; dMatson Evaluation of Social Skills with Youngsters; eParenting Practice Inventory.

(i.e., use of psychopharmacotherapy children, psychosocial treatment, family care etc.) received during the study. Participating families received €20,- for the first two home visits and €40,- for the third home visit. The Institutional Review Board in The Netherlands (METC UMC Utrecht, protocol number 11-320/K) approved the study.

Questionnaire measures

Parenting Practice Inventory (PPI)

The PPI measures parenting skills and discipline styles of parents with young children 6-12 years (Webster-Stratton, 2001a). The PPI consists of 15 sections, each containing multiple items, asking for a response of the parent to children’s misbehavior, appropriate behavior, and several statements. Parents answered these questions and responded to these statements using different scales. In total, four summary scales were extracted from this questionnaire: harsh and inconsistent discipline (15 items, e.g., ‘Threatening or threatening the child’), physical punishment (6 items, e.g., ‘Slapping or hitting when misbehavior occurs’), and praise and incentives (11 items, e.g., ‘Giving a hug or compliment’). Statements about parenting were excluded, because we were interested in the actual behavior of parents. To assess positive parenting behavior, we combined the dimensions positive verbal discipline and praise and incentives. To assess
Intervention Effectiveness of The Incredible Years: New Insights into Sociodemographic and Intervention-based Moderators

The observations were coded by trained research assistants who were not involved in the study and who were blind to condition and measurement wave. Monthly calibration meetings were held to prevent observer drift. To provide estimates of interrater reliability, a random 237 of the 1161 observations (20%) were independently coded by two coders. Coders were unaware of which observations were used to assess observer agreement. Intraclass correlation (ICCs) was assessed through a two-way mixed effects model using SPSS 22.0. Interrater reliability was excellent at all measurement waves; ICCs ranged from .96 to .97 for positive parenting, from .85 to .93 for negative parenting, from .82 to .93 for positive child behavior, and from .70 to .83 for negative child behavior.

Intervention: The Incredible Years BASIC program

The IY program is developed to prevent and intervene in the development of child externalizing behavior by building a warm parent-child relationship through increasing positive parenting strategies such as child directed play, social and emotion coaching, praise and incentives and decreasing negative parenting strategies such as being critical and inconsistent (Webster-Stratton, 2001b). IY is a group behavioral parent training program consisting of 15 weekly sessions. The program starts with the focus on positive parenting strategies such as play, praise and incentives, before discussing effective limit setting, ignoring unwanted behavior, and finally time out strategies. During the sessions parents watch video-vignettes of parents and children interacting (in our study Dutch subtitles were used in the vignettes), act in role-plays, have brainstorm sessions, and exchange experiences and ideas in small groups. After each vignette, the group leader asks questions to stimulate discussion about what parents found particularly (in)effective and to practice alternative responses. Parents are encouraged to role-play new skills in front of the group and in small subgroups during the meeting. Before each session, parents read a book chapter on the topic of that particular session. Additionally, they receive home assignments to practice the discussed skills at home. Parents are also assigned a “buddy” (i.e., another parent of the same group), which they call weekly to check in with and discuss successes and difficulties with the new learned skills. The program uses a collaborative setting, in which group leaders establish themselves as facilitators rather than as experts. Group leaders encourage parents to solve problems and to help each other solve problems in order to ensure maintenance of the intervention effects.

Fourteen IY intervention groups (consisting of 8-15 parents) were delivered across three different Dutch municipalities (i.e., large urban city, medium urban city, and a suburban area). The groups consisted of 14 weekly 2-hour sessions and a “booster” session one month after termination of the program (i.e., total of 15 sessions). Every group was led by two group leaders who had followed a commensurate three-day basic training. All main leaders had a background in clinical child psychology, had experience running IY groups before the study commenced, and were officially certified by The Incredible Years, Inc. (two group leaders got certified during the study). Parents completed weekly satisfaction questionnaires to ensure the session contents address the specific goals parents have. To boost attendance, child-care was arranged for parents who attended the course during office hours. Parents were compensated for travel costs when needed. At the start of the program all parents received the program book and an IY magnet. During the sessions group leaders provided the groups with coffee, tea, and snacks. Candy or stickers (parent’s choice) were handed out during the sessions as a reward for active participation. During the sessions on ‘tangible rewards’ leaders also brought small surprise rewards for parents (worth approximately 1 euro, such as stickers, stamps, or small games, which they had to bindly grab from a ‘treasure bag’). At the last session, all parents received a certificate, a personal felicitation talk, and a plant as a reminder of the program.

Analyses

Preliminary analyses

The preliminary analyses showed that there were no outliers but that the data were skewed. Therefore, we used maximum likelihood robust (MLR) to deal with non-normally distributed data. Also, two-level MxN models were run as preliminary analyses to test the nesting of families within intervention groups (i.e., families formed a part of fourteen groups). There was no variance at the group level at pretest, and at posttest variance did not exceed 4% of total variance. Therefore, group-level was not included in the final models.
Main analyses

In the primary analyses, latent growth curve modeling (LGCM) in Mplus (Muthén & Muthén, 2008-2015) was used to assess the development of observed and reported parenting and child behavior across pretest, posttest, and follow-up assessments. LGCM estimates individual growth for each child or parent separately, which is an excellent approach for examining variation in the development of the outcome variables, while controlling for baseline levels. Model fit is considered good if the Root Mean Square Error of Approximation (RMSEA) is < .05 and Confirmatory Fit Index (CFI) values are > .95 (Hu & Bentler, 1999). To calculate effect sizes of the intervention effectiveness Cohen’s $d$ was used, where $d > .20$ is considered a small effect, $d > .50$ as a moderate effect and $d > .80$ as a large effect. In total, 197 parents were assigned to the IY intervention groups, of whom 44 decided not to participate in the program or never attended a session. We found no differences on any pretest measures ($p > .09$) between parents who actively participated in the intervention and parents who did not. Therefore, the 44 allocated-to-intervention families who did not attend any sessions were still included in the analyses. In total, we assessed eight separate outcome measures (i.e., four parent-reported and four observed) in the full intention-to-treat analyses. Benjamini-Hochberg False Discovery Rate correction (Benjamini & Hochberg, 2005) was used to correct for chance capitalization.

After assessment of the intervention effectiveness, moderator variables (i.e., initial severity of externalizing behavior, child gender, SES measures by parental education, family composition, and number of sessions parents attended) were assessed using eight multivariate mixture models, controlling for possible confounding effects of the moderator variables. The effects of the moderator variables on the slope of the outcome variables were estimated for the two classes (intervention and control) separately, since the variable ‘number of sessions attended’ is only relevant in the intervention class.

RESULTS

Intervention integrity

A total of 197 parents were assigned to the IY intervention groups. Active participants attended on average 11.01 ($SD = 3.69$) out of 15 sessions. Of these participants, 74% attended at least 10 sessions and 84% at least half of the sessions. If parents missed a session, group leaders called them to discuss the content and sent them home assignments. If parents missed three subsequent visits, a home visit was scheduled by the trainers to discuss the missed program content with the parent(s). Besides the IY intervention about a third of the families in the experimental condition (31.30%) received additional (mental health) care or help between baseline and posttest, which was significantly less than families in the control condition (49.58%) ($\chi^2 (1, N = 350) = 3.71, p = .05$). The IY program can only be implemented by officially certified group leaders. To ensure and monitor implementation fidelity group leaders followed a treatment manual for each session (i.e., standardized manuals and session guidelines, check-lists, books, DVDs, handouts, tangible rewards etc.) and completed weekly protocol checklists of standards (e.g., vignettes, brainstorming, role-plays) to be covered in each session. Checklists showed that on average 70.4% of the standards were executed by trainers. It has been suggested that that positive intervention effects are often obtained with levels of program integrity exceeding 60% (Durlak & DuPre, 2008). Furthermore, co-leaders filled out standardized peer-feedback forms and parents filled out standardized feedback forms. All sessions were videotaped for feedback. Group leaders received ongoing supervision, feedback, and training throughout the study. Treatment integrity of IY overall is very high because of the close monitoring, standardized materials, and comprehensive training manuals (see Webster-Stratton & Hammond, 1997).

Table 4. Intervention Effects of Reported and Observed Child and Parent Behavior

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Intercept Effect</th>
<th>Slope Effect</th>
<th>Corrected Effect Sizes</th>
<th>p-value</th>
<th>RMSEA</th>
<th>CFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported positive behavior</td>
<td>.055 (.06)</td>
<td>-.186 (.04)***</td>
<td>&lt;.001</td>
<td>0.49</td>
<td>0.45</td>
<td>6.41 (2) 0.99</td>
</tr>
<tr>
<td>Reported negative behavior</td>
<td>.073 (.06)</td>
<td>-.175 (.04)***</td>
<td>&lt;.001</td>
<td>0.29</td>
<td>0.25</td>
<td>18.25 (4) 0.95</td>
</tr>
<tr>
<td>Observed prosocial behavior</td>
<td>.012 (.06)</td>
<td>.008 (.06)</td>
<td>.90</td>
<td>0.06</td>
<td>0.02</td>
<td>7.12 (4) 0.94</td>
</tr>
<tr>
<td>Reported prosocial behavior</td>
<td>.012 (.06)</td>
<td>.019 (.03)</td>
<td>.62</td>
<td>0.08</td>
<td>0.07</td>
<td>0.57 (2) 1.00</td>
</tr>
<tr>
<td>Observed externalizing behavior</td>
<td>.122 (.08)*</td>
<td>-.091 (.07)</td>
<td>.28</td>
<td>0.06</td>
<td>0.02</td>
<td>3.77 (2) 0.96</td>
</tr>
<tr>
<td>Reported externalizing behavior</td>
<td>.085 (.05)</td>
<td>-.125 (.04)**</td>
<td>&lt;.01</td>
<td>0.20</td>
<td>0.08</td>
<td>8.08 (2) 0.98</td>
</tr>
</tbody>
</table>

Note. df = degree of freedom; CFI = comparative fit index; RMSEA = root mean square error of approximation. As $p < 0.05$, the CFI is set to 1.0 and RMSEA to .001, which makes it sufficient to read off whether the p value is not significant. p values are corrected with Benjamini-Hochberg False Discovery Rate correction. *p < .05. **p < .01. ***p < .001.
Intervention effects

Child externalizing behavior

For parent-reported child externalizing behavior on the ECBI intensity scale, condition proved non-significant at intercept ($B_0 = .085, p = .12$) but proved significant at slope ($B_1 = -.125, p = .001$, corrected $p = .002$) ($t^2 [df = 2, N = 387] = 8.08, CFI = .98$, RMSEA = .09, $d = .08$), indicating that reported levels of child externalizing behavior did not differ between control and intervention group at pretest, but that parents in the intervention group reported a significantly larger decrease in child externalizing behavior at posttest and follow-up compared to parents in the control group (see Table 4). For observed child externalizing behavior, after correction for multiple testing, condition proved neither significant at intercept ($B_0 = .122, p = .12$) nor at slope ($B_1 = -.091, p = .18$, corrected $p = .28$) ($t^2 [df = 2, N = 382] = 3.77, CFI = .96$, RMSEA = .05, $d = .02$), indicating that observed levels of child externalizing behavior between the intervention group and control group did not significantly differ at pretest or over time.

Child prosocial behavior

For parent-reported child prosocial behavior, condition proved neither significant at intercept ($B_0 = .012, p = .85$) nor slope ($B_1 = .019, p = .54$, corrected $p = .62$) ($t^2 [df = 2, N = 387] = 0.57, CFI = .100$, RMSEA < .001, $p = .75$, $d = .07$). Also, for observed child prosocial behavior, condition proved neither significant at intercept ($B_0 = .012, p = .85$) nor slope ($B_1 = .008, p = .90$, corrected $p = .90$) ($t^2 [df = 4, N = 382] = 7.12, CFI = .94$, RMSEA = .05, $d = .02$). Thus, reported and observed levels of child prosocial behavior between the intervention group and control group did not significantly differ at pretest or over time (see Table 4).

Negative parenting behavior

For parent-reported negative parenting behavior condition proved non-significant at intercept ($B_0 = .073, p = .23$) but proved significant at slope ($B_1 = -.175, p < .001$, corrected $p < .001$) ($t^2 [df = 4, N = 387] = 18.25, CFI = .95$, RMSEA = .10, $d = .25$) (see Table 4), indicating that the control and intervention group did not differ on negative parenting behavior at pretest, but that parents in the intervention group reported a significantly stronger decrease of negative parenting behavior over time compared to the control group. For observed negative parenting behavior, condition proved neither significant at intercept ($B_0 = -.087, p = .40$) nor slope ($B_1 = -.063, p = .44$, corrected $p = .58$) ($t^2 [df = 4, N = 382] = 6.35, CFI = .98$, RMSEA = .04, $d = .02$), indicating that change in observed levels of negative parenting behavior did not significantly differ between groups.

Positive parenting behavior

For parent-reported positive parenting behavior condition proved non-significant at intercept ($B_0 = .055, p = .38$) but significant at slope ($B_1 = .186, p < .001$, corrected $p < .001$) ($t^2 [df = 2, N = 387] = 6.41, CFI = .99$, RMSEA = .08, $d = .45$) (see Table 4). Also, for observed positive parenting behavior, condition proved not significant at intercept ($B_0 = .130, p = .17$) but significant at slope ($B_1 = .280, p < .001$, corrected $p < .001$) ($t^2 [df = 4, N = 382] = 16.93, CFI = .96$, RMSEA = .09, $d = .02$). Thus, parent-reported and observed positive parenting behavior did not differ at pretest, but parents in the intervention group reported and showed a significantly stronger increase of positive parenting behaviors over time compared to parents in the control group.

All significant effects survived correction for multiple testing. In addition, we also performed a “completers-only” analyses (i.e., only including families who attended at least one IY session). The results remained the same as the results of the intention-to-treat analyses (see Supplementary Material, Table A).

Moderators of intervention effects

Table 3 shows correlations between the moderator variables. Of these variables, initial severity of externalizing behavior was significantly negatively correlated with child gender ($r = -.21, p < .05$) and family composition ($r = -.21, p < .05$), indicating that single...
parents and parents of boys reported more initial severity of externalizing behavior. SES was significantly negatively correlated with family composition ($r = - .13, p = .01$) and positively correlated with number of sessions parents attended the intervention group ($r = .16, p = .05$), indicating that low SES families included more single parents and attended less intervention sessions when allocated to the intervention group. As planned, these correlations between moderator variables were taken into account to control for their possible mutually confounding effects.

**Initial severity of externalizing behavior**

Initial severity of child externalizing behavior was a significant predictor of the slope of parent-reported externalizing behavior over time in the intervention group ($B_0 = .015, p = .85$), indicating that girls in the intervention group showed a larger effect of the intervention on externalizing child behavior over time (see Table 5). However, comparison of the coefficients of the control and intervention group showed, that the coefficients were not significantly different from each other ($t = 1.15, df = 374, p = .25$). Initial severity did not influence intervention effects on observed child externalizing behavior, reported and observed child prosocial behavior, reported and observed negative parenting behavior, and reported and observed positive parenting behavior. Thus, no moderation of intervention effects by initial severity was found.

**Child gender**

Child gender predicted the slope of observed child prosocial behavior over time in the intervention group ($B_0 = .015, p = .85$), indicating that girls in the intervention group showed a larger increase in prosocial behavior compared to boys in the intervention group. However, comparison of the coefficients of the control and intervention group showed, that the coefficients were not significantly different from each other ($t = 2.77, df = 374, p = .19$). Child gender did not influence intervention effects on reported and observed child externalizing behavior, reported child prosocial behavior, reported and observed negative parenting behavior, and reported and observed positive parenting behavior. Thus, no moderation of intervention effects by child gender was found.

**SES**

SES (parental education) was a predictor of the slope of reported negative parenting behavior in the control group. Low SES families in the control group reported more negative parenting over time ($B_0 = .087, p = .05$) but there was no such effect of SES on slope of reported negative parenting in the intervention group ($B_0 = .005, p = ns$). However, comparison of the coefficients of the control and intervention group showed, that the coefficients were not significantly different from each other ($t = 1.45, df = 364, p = .15$). Also, we found that SES was a predictor of the slope of observed child externalizing behavior in the control condition ($B_0 = -.156, p = .04$) but not in the intervention condition ($B_0 = .000, p = .00$). This indicates that in the control condition higher SES predicted lower levels of child externalizing behavior over time. However, comparison of the coefficient of the control and intervention group showed, that the coefficients were not significantly different from each other ($t = 1.56, df = 374, p = .12$). In addition, SES was a predictor of the slope of observed child prosocial behavior in the intervention condition ($B_0 = .000, p = .000$) but not in the control condition ($B_0 = -.020, p = ns$). However, comparison of the coefficients of the control and intervention group showed, that the coefficients were not significantly different from each other ($t = 1.16, df = 374, p = .15$). Thus, no moderation of intervention effects by SES was found.

**Family composition**

Family composition was not a significant predictor of the slope of any of the outcomes, indicating that single parent and two-parent families benefited equally from the IY intervention.

The number of intervention sessions parents attended

Number of attended IY sessions predicted the slope of parent-reported negative parenting behavior ($B_0 = -.012, p = .001$), parent-reported positive parenting behavior ($B_0 = .011, p = .001$), and observed positive parenting behavior ($B_0 = .018, p = .001$) in the intervention group. These results indicate that parents who attended more IY sessions reported a higher decrease in negative and higher increase in positive parenting behavior and also showed a larger increase in observed positive parenting behavior than parents who attended less IY sessions. The number of intervention sessions parents attended did not influence intervention effects on reported and observed child externalizing behavior, reported and observed prosocial child behavior, reported positive parenting behavior, and observed negative parenting behavior.

**DISCUSSION**

Previous research demonstrated that the behavioral parenting training (BPT) Incredible Years (IY) is effective in preventing externalizing behavior. In addition, studies suggest that specific sociodemographic and intervention-based factors (i.e., initial severity of externalizing behavior, child gender, social economic status (SES), family composition, and number of sessions attended) may influence the intervention effectiveness of the program. However, the effects of these moderators were mostly studied in isolation rather than in multivariate analyses, inhibiting insight into "real" moderation effects when different moderators are controlled for each other. Also, these effects were studied in modestly sized samples (e.g., average $N = 95$, Menting et al.,
Intervention Effectiveness of The Incredible Years: New Insights into Sociodemographic and Intervention-based Moderators

my dopamine has been busy      –      chapter      5

Intervention effects were neither found for observed child externalizing behavior and observed negative parenting behavior nor for reported and observed child prosocial behavior. Therefore, even though a recent meta-analysis found convincing proof that IY is effective in preventing observed child externalizing behavior (dad = .37 for all 23 studies, dad = .35 for the four indicative prevention studies; Menting et al., 2013), we did not establish this effect in the present study. In interpreting these findings it is important to note that reliability of the two observed child behavior scales was relatively low and that we adhered to stringent controls for multiple testing. This resulted in low power to detect effects on observed child behavior. We found no effects of IY on child prosocial behavior. This is contradictory to the outcomes of a recent meta-analysis by Menting and colleagues (2013), who found IY to be effective in reducing externalizing behavior as well as increasing child prosocial behavior. This might be partly explained by differences in how prosocial behavior is operationalized and assessed between studies.

Our results illustrate the importance of using multi-informant data when assessing intervention effects. Including both reported and observed data on parent and child behavior gave a more complete picture of changes herein. Specifically, although we found significant intervention effects on most parent-reported measures, on the observed measures we only found a significant effect on positive parenting behavior. It has been argued that parents might justify the time and effort they have invested through attending the intervention by reporting a decrease in child externalizing behavior, without the occurrence of an actual change in such behavior (Leijten, Overbeek, & Janssens, 2012). However, both the recent Menting and colleagues (2013) meta-analysis, including 23 studies with observational measures, and a Dutch study by Posthumus and colleagues (2012) did establish effects on observed child behavior. Among those studies the DPICS is often used as observation instrument, however there is little correspondence between studies in which DPICS categories for parent and child behavior are being used. For instance, some studies used a composite score of the categories child smart talk, Cry/Whine/Yell, and physical negative, excluding (Posthumus et al., 2012) or including non-compliance (Webster-Stratton, Reid, & Hammond, 2001) and destructive behavior (Webster-Stratton, 1998), whereas others used separate categories such as non-compliance or physical negative to index child externalizing behavior (Eyberg et al., 2001). It might be that previous studies also encountered difficulties in forming a reliable scale for this behavior. In our case, inability to create a reliable scale for observed child behavior combined with small variance might have precluded detection of an intervention effect in the present study. Another possible explanation might be differences between the parenting questionnaire and observation measure. The questionnaire asks parents about child behavior over a longer period of time and across different contexts, whereas the observation is conducted in a period of 20 minutes and is restricted to a play-session. Future research could explore whether different effects can be captured with observations using a more extensive timeframe and/or in different settings (e.g., morning routines or mealtimes).

The discrepancy between specifically our findings on observed positive parenting behavior and observed negative parenting behavior, might also suggest that the increase in positive parenting behavior is the most rapidly evolved and/or most robust intervention induced behavioral change. This seems plausible, considering that the IY program highly invests in strengthening the parent-child relationship by advocating positive parenting strategies (such as regular playtime and praise). Furthermore, it might be easier to stimulate parents to increase the use of novel positive parenting behaviors than to change coercive parent-child interaction patterns. In addition, it may be that, although parents already perceive and report a decrease in their own negative parenting behavior and their child’s externalizing behavior, these behaviors have not yet observably changed enough to be detected by the limited observation measure at the time of the follow-up.

The simultaneous inclusion of multiple potential moderators in our analyses proved worthwhile, as moderators that have previously been studied in isolation were found to be correlated. Initial severity of externalizing behavior and number of sessions parents attended were the only moderators that influenced significant IY intervention effects. However, we did not find a consistent pattern of moderation across all outcome measures examined. For example, the number of sessions that parents attended moderated the intervention effects on reported negative parenting behavior and reported observed positive parenting behavior, but not on any child behavior outcome measures. Out of 40 tested moderation effects (i.e., eight outcomes × five moderators), we eventually found three significant moderation effects. This raises the question how potent these independent moderators are in differentiating the effectiveness of the intervention, and consequently, the true clinical relevance of these moderators. The current findings indicate that IY may be an effective intervention, specifically for reducing perceived externalizing child behavior, across a broader range of child and family subgroups.

However, our findings do not rule out that the intervention effects are indeed different for different children and families and/or are influenced by moderators other than the ones currently examined. For example, the operationalization of parenting may...
be culture-bound, that in that parenting practices related to negative child behavior may differ by ethnicity. The current study had a homogenous sample for which the number of parents not born in the Netherlands was only 16% and the number of parents not born in a Western-European country was less than 13%. As our sample was a predominantly indigenous Dutch sample we were unable to examine ethnicity as a possible moderator. On the other hand, a previous study on IY, targeting Dutch ethnic minority mothers, showed that ethnic minorities benefited equally from IY compared to Dutch families (Leijten, Raaijmakers, et al., 2015). Furthermore, a more theoretically informed search for moderators may be fruitful. We know that externalizing behavior is a very heterogeneous behavioral cluster and has different etiologies in different children (e.g., Frick, 1998). This suggests that intervention effects may depend on the extent to which a specific intervention addresses the specific factors pertinent to the development of these behaviors in individual children. Thus, interesting moderators to investigate might be those factors that are likely to make children more or less susceptible to specific intervention techniques. Some of these factors indicated by previous studies relate to the neurocognitive domain such as inhibitory control (Lochman et al., 2015; Matthis et al., 2012), to child temperament (e.g., Galitto, 2015; Scott & O’Connor, 2012), and to children’s genetic make-up (Bakermans-Kranenburg & Van Uzendoorn, 2015). More insight into “what works for whom” might also help to tailor interventions and to improve their effectiveness.

Using a full intention-to-treat model, the overall effect sizes of the intervention were small ranging between $d = .06$ and $d = .49$ at posttest and $d = .02$ and $d = .45$ at follow-up. However, the effect size on reported externalizing child behavior at follow-up was comparable to meta-analytical findings on the effectiveness of IY in an indicated prevention context (Menting et al., 2013). Another way to further improve effectiveness of prevention programs is by unveiling the active components why interventions are effective (i.e., mediators). One way to gain more insight into such mechanisms of change, is by conducting micro trials (i.e., small-scale randomized experiments using a brief and focused environmental manipulation, designed to target one specific risk mechanism) focused on discrete parenting intervention elements (Collins, Murphy, & Strecher, 2007; Howe et al., 2010; Leijten, Dishion, et al., 2015).

Our findings have to be interpreted in the light of some limitations. First of all, because of the indicated prevention setting we had a large group of parents who were attributed to the intervention but never participated. We used an intention-to-treat model to estimate more realistic intervention effects of IY in a real-world outreaching prevention setting, including those parents allocated to the intervention that did not participate. However, as such an analysis might lead to a conservative estimation of intervention effects we also conducted “completers-only” analyses that showed similar intervention effects (see Supplementary Material). Secondly, our follow-up was on average only 4 months after the intervention. Therefore, we cannot say whether the improvements in parenting and child behavior remain over a longer period of time. However, previous longitudinal and quasi experimental studies do suggest long-term effects of IY up to adolescence (Jones, Daley, Hutchings, Bywater, & Eames, 2008; Posthumus et al., 2012; Webster-Stratton, Rinaldi, & Reid, 2011). More experimental research is needed to confirm these long-term findings, but also to further investigate the longitudinal role of the sociodemographic and intervention-based moderators. Third, IY is a highly protocolled program, which overall yields high treatment integrity (Webster-Stratton & Hammond, 1997). Following IY standard procedure, program integrity is measured using group leader self-reported checklists. However, the use of therapist-reported treatment integrity has been criticized (Perepletchikova & Kazdin, 2005). It might be that other measures of integrity (e.g., observational coding of sessions) would yield lower integrity scores. Fourth, our study also only included a small number of single parent and low SES families, which might cause a power issue to detect possible moderator effects of family composition and low generalizability to other samples (see Table 1). Finally, it is worth mentioning that the reliability of observed child behavior was low ($\alpha = .49$ to .67), which could have led to less reliable estimations of intervention effects. Nonetheless, for reasons of transparency, we decided to report the analyses because they were conducted to test previously published hypotheses (see for a priori hypotheses in Changurat, Weeland et al., 2012).

Despite these limitations, our current trial may be considered a major step forward.
in terms of its large scale, the use of observational data to establish intervention effects on child and parenting behavior, the use of sophisticated statistical analyses, controlling for multiple testing, and high level of attendance at the intervention meetings and little overall attrition (retaining 93% of participants at follow-up). Moreover, we sought to extend recent work in this area by examining multiple moderators indicated by previous meta-analyses and by investigating the unique effects (i.e., controlling for possible confounding effects of different moderators) of these moderators on the intervention effectiveness. Our result show that previously suggested moderators may not be as potent in differentiating BPT effects as once thought. Based on this approach, IY has proven to be an effective prevention strategy to reduce parent perceived child externalizing behavior in a prevention setting.

**FUNDING**

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**ACKNOWLEDGEMENTS**

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**SUPPLEMENTARY MATERIAL**

Table 1a.
GENETIC MODERATION OF INTERVENTION EFFICACY: Dopaminergic Genes, The Incredible Years, and Externalizing Behavior in Children
CHAPTER 6
GENETIC MODERATION OF INTERVENTION EFFICACY: DOPAMINERGIC GENES, THE INCREDIBLE YEARS, AND EXTERNALIZING BEHAVIOR IN CHILDREN

KEYWORDS – Externalizing behavior
Gene-by-environment interaction
Dopaminergic plasticity


ABSTRACT – This study investigated whether children scoring higher on a polygenic plasticity index based on 5 dopaminergic genes (DRD4, DRD2, DAT1, MAOA, and COMT) benefited the most from The Incredible Years (IY) parent program. Data were used from a randomized-controlled-trial including 341 Dutch families with 4-8-year-old children (55.7% boys) showing moderate-to-high levels of problem behavior. IY proved most effective in decreasing parent-reported (but not observed) externalizing behavior in boys (but not girls) carrying more rather than fewer dopaminergic plasticity alleles; this gene×intervention effect was most pronounced in the case of boys whose parents' manifested the most positive change in parenting in response to the intervention. These results proved robust across a variety of sampling specifications (e.g., intention to treat, ethnicity).

Elevated levels of externalizing behavior (e.g., aggression, oppositional behavior, disobedience) in the early years of life might forecast a variety of problems later in childhood (Campbell et al., 2006). Left untreated, externalizing behavior often worsens with age and tends to persist over time (Mesman, Bongers, & Koot, 2001; Vaughn et al., 2013), generating substantial social and economic costs to individuals and society (Raaijmakers et al., 2011; Scott et al., 2001). These observations underscore the importance of early intervention to ameliorate such early emerging problems. Some of the most effective interventions in this regard are designed to increase positive parenting behavior. Such behavioral parent training programs employ parents as change agents, enabling them to deploy more positive parenting practices, thereby reducing problematic child behavior (Forehand, LaFko, Parent, & Burt, 2014).

Not all children benefit equally from intervention-induced changes in positive parenting behavior. Conversely, not all children who appear to be at risk for developing externalizing problems—as a result of exposure to unsupportive, negative and harsh parenting—do so. These differential responses to, respectively, parenting support and risk raise questions about the source of such heterogeneity. For quite some time it has been presumed that some children are more at risk because of their own characteristics of “vulnerability,” be they temperamental, physiological and/or genetic in nature. Indeed, this notion is central to the long-standing diathesis-stress model of person×environment interaction which has informed much research (e.g., Zuckerman, 1999). However, this “vulnerability” framework is less adequate in explaining heterogeneity in response to environmental support and enrichment.

Recently, an alternative person×environment framework has been advanced which explicitly addresses differential response to both risk and support. This differential susceptibility perspective stipulates that the very personal characteristics that make a child especially vulnerable to adversity may also enable him or her to benefit more so than others from support and enrichment (Belsky et al., 2007, Belsky & Pluess, 2009, 2013, Boyce & Ellis, 2005). In so doing, this evolutionary inspired framework implies that it will be children regarded as especially vulnerable to adversity due to their personal characteristics who would benefit the most from efforts to promote well-being, to prevent problems from developing in the first place, and to ameliorate existing problems (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015).

Here we test this differential-susceptibility-derived proposition that some children are more susceptible to intervention-induced environmental change than others by focusing on their genetic make-up. Specifically, we test the hypothesis that children manifesting moderate-to-high levels of problem behavior and carrying more of particular variants of a set of dopaminergic genes (i.e., “plasticity alleles”) will benefit more than their peers from The Incredible Years (IY) parenting intervention (Webster-Stratton, 2001b). This prediction is also based on recent research on the genetic moderation of intervention efficacy (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015).
Genetic Moderation of Intervention Efficacy: Dopaminergic Genes, The Incredible Years, and Externalizing Behavior in Children

Even having minimal but detectable signs or symptoms (d = .20, Menting et al., 2013). Even though the effects might be small, the impact of preventive intervention programs with children varying in the degree to which they benefit from the program. In fact, a recent meta-analysis of IY effectiveness chronicled a substantial effect in treatment of children with severe externalizing behavior problems or to ameliorate early emerging problems and is therefore the specific focus of this report. IY has been evaluated in over 50 studies of severe externalizing behavior have targeted parenting, seeking to reduce negative, while promoting positive parenting practices. IY parent training (IY; Webster-Stratton, 2001b) is one such program designed to prevent the development of child externalizing behavior problems or to ameliorate early emerging problems and is therefore the specific focus of this report. IY has been evaluated in over 50 studies and proven effective in both clinical and community samples (Menting, Orobio de Castro, & Matthys, 2013; Webster-Stratton & Hammond, 1997; Weeland, Chhangeur et al., in press). IY intervention effects have been replicated independently (e.g., Scott, Spender, Doolan, Jacobs, & Aspland, 2001) and results include reductions in externalizing behavior that endure several years after exposure to the program (e.g., Jones et al., 2008; Posthumus et al., 2012).

Important to appreciate, however, is that average effect size of IY and most other parenting interventions are small to moderate in magnitude (McCurt et al., 2006), with children varying in the degree to which they benefit from the program. In fact, a recent meta-analysis of IY effectiveness chronicled a substantial effect in treatment studies, but a small effect in indicated prevention research on children identified as having minimal but detectable signs or symptoms (d = .20, Menting et al., 2013). Even the most successful interventions for externalizing behavior might be effective for only about two thirds of children (see Webster-Stratton & Hammond, 1997). Such results highlight the need to illuminate potential moderators of intervention efficacy. It seems plausible that such determinants of variation in response to intervention could be genetic in character, as responsiveness to changes in parenting may depend on reward sensitivity. Specifically, the dopaminergic system would seem to play a pivotal role due to its link with reward sensitivity and reinforcement learning (Bakermans-Kranenburg & Van Uzendoorn, 2011).

Parenting and externalizing behavior

Extensive evidence indicates that parenting behavior is longitudinally associated with child behavior (e.g., Miner & Clarke-Stewart, 2008). Negative parenting strategies in particular are related to elevated levels of externalizing behavior, including inconsistent discipline, disapproval, harshness, physical discipline, lack of positive tone, and coercion (Gershoff, 2002; Pettit & Bates, 1989; Shaw et al., 1994). In contrast, positive parenting strategies that convey warmth and acceptance and provide positive consequences for desirable behavior while enhancing the parent-child relationship can prevent externalizing behavior from persisting and increasing over time ( Dishion et al., 2009; Gardner, Shaw, Dishion, Burton, & Supplee, 2007).

The Incredible Years program

Given these observations, it is not surprising that efforts to prevent the development of severe externalizing behavior have targeted parenting, seeking to reduce negative, while promoting positive parenting practices. IY parent training (IY; Webster-Stratton, 2001b) is one such program designed to prevent the development of child externalizing behavior problems or to ameliorate early emerging problems and is therefore the specific focus of this report. IY has been evaluated in over 50 studies and proven effective in both clinical and community samples (Menting, Orobio de Castro, & Matthys, 2013; Webster-Stratton & Hammond, 1997; Weeland, Chhangeur et al., in press). IY intervention effects have been replicated independently (e.g., Scott, Spender, Doolan, Jacobs, & Aspland, 2001) and results include reductions in externalizing behavior that endure several years after exposure to the program (e.g., Jones et al., 2008; Posthumus et al., 2012).

Important to appreciate, however, is that average effect size of IY and most other parenting interventions are small to moderate in magnitude (McCurt et al., 2006), with children varying in the degree to which they benefit from the program. In fact, a recent meta-analysis of IY effectiveness chronicled a substantial effect in treatment studies, but a small effect in indicated prevention research on children identified as having minimal but detectable signs or symptoms (d = .20, Menting et al., 2013). Even the most successful interventions for externalizing behavior might be effective for only about two thirds of children (see Webster-Stratton & Hammond, 1997). Such results highlight the need to illuminate potential moderators of intervention efficacy. It seems plausible that such determinants of variation in response to intervention could be genetic in character, as responsiveness to changes in parenting may depend on reward sensitivity. Specifically, the dopaminergic system would seem to play a pivotal role due to its link with reward sensitivity and reinforcement learning (Bakermans-Kranenburg & Van Uzendoorn, 2011).

Differential susceptibility to environmental influences

Decades of research informed by the diathesis-stress model of person-environment interaction (Zuckerman, 1999) made clear that some individuals are more susceptible to the negative effects of exposure to diverse conditions of contextual adversity (e.g., poverty, depressed mother, harsh parenting); and that this enhanced vulnerability to adversity might be a function of their personal characteristics (i.e., temperamental, physiological or genetic characteristics). Recent theorizing has called attention to the fact that the very individual attributes that appear to make some children more vulnerable to adversity might also make them more likely to benefit from supportive environmental conditions. Indeed, differential-susceptibility theorizing postulates that some children are more developmentally plastic or malleable “for better and for worse” rather than just more likely to succumb to negative rearing conditions (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005).

A growing body of evidence is consistent with this claim (Belsky & Pluess, 2009, 2013). Especially important for purposes of the current study is research documenting the role of dopaminergic genes in moderating a variety of environmental effects in a for-better-and-for-worse, differential-susceptibility-related manner. For example, Foley and colleagues (2004) found that boys with the MAOA low-activity allele were more likely than their high-activity counterparts to be diagnosed with conduct disorder when exposed to high levels of childhood adversity, but were less likely to do so when exposed to low levels of adversity. The polymorphisms selected for inclusion in the work reported herein have been found to moderate environmental effects in gene by environment (G × E) research in a similar differential-susceptibility-related manner (Belsky & Pluess, 2013; Van Uzendoorn & Bakermans-Kranenburg, 2015). The current investigation is therefore specifically conceptualized in such terms rather than in terms of vantage sensitivity—which refers to factors that make some individuals more susceptible to positive exposures but does not make them more susceptible to negative ones (Pluess & Belsky, 2013).

Why dopaminergic genes?

Dopamine is an excitatory neurotransmitter involved in motivational, attentional, and reward processes. It is heavily expressed in dopaminergic pathways in the brain.
(e.g., the ventral tegmental area, nucleus accumbens, and prefrontal cortex) where it appears to modulate excitatory signaling (Blum et al., 1996). This signaling plays an important role in reward processing. Different dopaminergic polymorphisms are known to alter reward processing—that makes children and adolescents apparently more or less prone to environmental cues of reward by affecting how much dopamine moves into a synapse or how quickly it is reabsorbed or degraded (Matthys et al., 2013; Moore & Depue, 2016). This might influence how susceptible children are to parenting practices based on reward. Here we focus on the cumulative function of several polymorphisms that are known to affect levels of dopamine signaling in the brain, and thereby possibly contribute to individual differences in reward processing: the 7-repeat allele of DRD4, the A1 allele of DRD2, the 10-repeat allele of DAT1, the low-activity allele of MAOA, and the val allele of COMT.

All the just-listed allelic variants have been linked to increased sensitivity to environmental influences in a for-better-and-for-worse manner (Belsky & Pluess, 2009, 2013). Consider in this regard evidence of Laucht and colleagues (2007) showing that adolescents carrying the DAT1 10-repeat allele manifested most and least inattentiveness problems when living under high and low aversive conditions, respectively, compared to other children. Relatedly, Kim-Cohen and colleagues (2006) found that boys carrying the MAOA low-activity allele were rated by teachers and mothers as having more mental health problems than other boys when experiencing physical abuse, but fewer problems when not mistreated. Notably, in fact, is Bakermans-Kranenburg and Van IJzendoorn’s (2011) meta-analysis showing that individual dopamine-related genes moderate diverse environmental effects in a differential-susceptibility-related manner in the case of children under 10 years of age. Just as noteworthy, however, is that G × E results of correlational studies have proven difficult to replicate and the interpretation of G × E effects has not always been straightforward (e.g., Duncan & Keller, 2011).

**Gene × Intervention interaction**

A major step forward in testing G × E builds on an experimental paradigm that involves intervention. As such, G × I (gene-by-intervention) research has several advantages (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015). First, it eliminates possible gene-environment correlations (G × E) that plague interpretation of virtually all G × E work (Bakermans-Kranenburg & Van IJzendoorn, 2015; Chilingerian, Weeland, Matthys, & Overbeek, 2015). Second, G × I research provides a means for establishing differential susceptibility due to its focus on environmental enrichment. Third, standardized interventions afford precise and thus reliable measurement of the environment, thereby reducing measurement error. Fourth, G × I designs provide considerably more statistical power due to the dichotomous parameterization of an environmental factor (i.e., experimental/control), as well as a targeted focus on “at risk” samples (Bakermans-Kranenburg & Van IJzendoorn, 2015).

Research on genetic moderation of intervention efficacy is growing following a pioneering study showing that video-feedback intervention designed to reduce externalizing behavior by promoting sensitive parenting and positive discipline proved effective only for children carrying 7-repeat alleles of the DRD4 gene (Bakermans-Kranenburg et al., 2008). More recent research further revealed that children carrying the 7-repeat allele disproportionately benefited from computerized training designed to enhance phonemic awareness (Kegel, Bus, & Van IJzendoorn, 2011) and, separately, text comprehension (Plak, Kegel, & Bus, 2015). Working with older children—African-American adolescents growing up in rural Georgia—Brady, Yu, and Beach (2015) observed that a family-based intervention designed to prevent, among other things, substance use, proved effective principally for teenagers carrying this same putative plasticity allele. Most notably, Van IJzendoorn and Bakermans-Kranenburg (2015) meta-analysis found that genetic moderation of efficacy proved to be the norm, even across interventions of varying intensity (Van IJzendoorn & Bakermans-Kranenburg, 2015). One limitation of virtually all G × I work to date, however, is its focus single candidate genes, thus failing to do justice to the polygenic nature of development.

**Current study**

The research reported herein evaluates the genetic moderation of the efficacy of the IY program. Rather than focusing on a single candidate gene we employ a systems’ approach (Nikolaev et al., 2011), creating a dopaminergic polygenic composite (based on the allelic variants already highlighted). Following Belsky and Beaver (2011), we gave children one point for each polymorphism for which they had at least one putative plasticity allele (i.e., 0/1 scoring, range: 0-5). We predicted that children scoring highest on the polygenic index would show the greatest decrease in externalizing behavior in response to the IY intervention; and that this would be especially so when parents evinced substantial rather than limited improvement in their positive parenting behavior in response to intervention. In other words, it would be children carrying many rather than few plasticity alleles whose parents changed the most who would benefit most from the IY program. After evaluating these predictions with all children, we conducted a series of sensitivity analyses based on appreciation that (1) inclusion of children who vary in their race/ethnicity could be problematic when genetics are a focus of interest (Propper, Willoughby, Halpern, Carbone, & Cox, 2007) and (2) results can differ when only cases with complete data are studied vs. when an intention-to-treat design is employed. In all analyses, G × I effects were tested separately for boys and girls because one of the polymorphisms included in our polygenic index, the MAOA, is sex linked (Byrd & Manuck, 2014).
Genetic Moderation of Intervention Efficacy: Dopaminergic Genes, The Incredible Years, and Externalizing Behavior in Children

**METHODS**

**The ORCHIDS study**

Data for the research reported here comes from the ORCHIDS study (Observational Randomized Controlled Trial on Childhood Differential Susceptibility) conducted in The Netherlands. Data were collected in two cohorts from November 2012/2013 through November 2015/2014. Detailed information about the sample and sampling appears elsewhere (Chhangur, Weeland et al., 2012; Weeland, Chhangur et al., in press).

Using a randomized control design, the ORCHIDS study addresses the differential effectiveness—across children with varying temperamental and genetic characteristics—of the IY program in reducing externalizing behavior in 4-8-year-old children showing moderate-to-high levels of such problems by enhancing a warm parent-child relationship through child directed play, coaching of social, emotional, and academic skills, praise and rewards, effective limit setting, and handling (e.g., ignore and time-out techniques).

**Sample**

Participants were recruited in two cohorts via two Dutch regional health care organizations. Parents of 20,084 children aged 4-8 were mailed a questionnaire to assess frequency of externalizing child behavior (i.e., screening stage), resulting in 5,876 questionnaires returned in a timely manner (response rate: 22.5%). Children scoring at or above the 75th percentile on the Eyberg Child Behavior Inventory (ECBI) Intensity Score scale (n = 1,524) were eligible for the RCT. If parents reported moderate-to-high levels of externalizing behavior of multiple children within a family, the child with the highest ECBI Intensity score was invited. A total of 1,393 mother-child or father-child dyads were thus invited to participate and 61% of these to-be-recruited families were reached; 46% of the latter (N = 387) agreed to participate. ECBI Intensity scores from screened families (M = 2.65, SD = 0.52) differed from invited families (M = 3.59, SD = 0.46, t (5872) = -8.957, p < .001) and from those who agreed to participate (M = 3.64, SD = 0.47, t (5872) = -28.90, p < .001), though those who agreed to participate scored somewhat higher than those invited but did not participate (t (1522) = -2.54, p = .01).

Cheat cells were collected for DNA assaying from 385 children (failed genotyping assay; n = 2), with the primary-analysis sample consisting of 341 of these children and their parents. 44 families randomized to the intervention group are excluded from the primary analysis because they did not attend any of the intervention sessions. Notably, those excluded (M = 3.66) did not differ from those included on the ECBI Intensity score (M = 3.71, p = .56). Most parents participating in the RCT were mothers (89.4%), Caucasian (90.5%), and well educated (23.3% vocational training, 42.9% higher vocational training or university). Intervention and control groups did not differ on sample characteristics (i.e., age, child age, gender, number of siblings, educational level, ethnicity) and baseline externalizing behavior (see Table 1).

**Design and procedure**

Two months following screening trained research assistants conducted pretest home visits to collect DNA and observational data from the child, questionnaires to measure child externalizing behavior and positive parenting behavior were emailed to parents a week earlier. Subsequently, families were randomly assigned (ratio: 1:1) to control (n = 190) or intervention group (n = 197); recall that 44 families allocated to intervention did not attend any sessions, thus being excluded from the primary
analyses. Approximately four and eight months after the pretest (i.e., posttest, follow-up), parents again completed the questionnaires and observations were made of child externalizing behavior. The Institutional Review Board in The Netherlands (METC UMC Utrecht, protocol number 11-320/K) approved the study.

Incredible Years parent training

The IY program uses a collaborative group approach; group leaders serve as facilitators rather than experts while seeking to empower parents. Issues and topics addressed include the importance of child-directed play, social-and-emotion coaching, the use of praise to reward and incentives to motivate appropriate behavior, and the importance of consistency in the use of non-corporal disciplinary practices. This trial involved 14 weekly sessions in which parents watched and discussed video-vignettes of parent-child interactions, engaged in role playing, and discussed family experiences in small (sub)groups. More specifically, following each vignette, group leaders asked questions to stimulate discussion about (in)effective parenting behavior and alternative approaches. Before sessions, parents received exercises to practice at home, read relevant literature, and practiced behavior-management skills with their child. A final and 15th session took place a month following the 14th session, serving as a “booster” to consolidate intervention effects by repeating, discussing, and practicing skills. The intervention groups consisted of 8-15 parents. Parents attended an average of 11 of the 15 sessions (M = 10.85, SD = 3.95). Although the parent who filled out the questionnaires attended the sessions, the other parent was allowed to do so as well. Every group was led by two group leaders. Main leaders had a background in clinical child psychology, had experience running IY groups before the study commenced, and were officially certified group leaders. All leaders received two-hour supervision sessions at least three times across the 14-week period. Besides these, regular inter-session meetings between group leaders took place (see also Weeland, Chhangur et al., in press).

Measures

Parent-reported externalizing behavior

The Eyberg Child Behavior Inventory (ECBI; Eyberg & Pincus, 1999), used at screening, pretest, posttest, and follow-up, assessed child externalizing problems. One of the instrument’s two scales is the focus of in this report. This Intensity subscale consists of 15 scales, each containing several items related to how parents typically respond to their child’s appropriate and inappropriate behavior (measured using a 7-point Likert-type scale: 0 = not likely/never to 7 = very likely/always). For this study, praise and incentives (11 items) and positive verbal discipline (9 items) were combined to create an index of positive parenting behavior (e.g., “When my child complete chores I praise him,” “When my child refuses do to something I discuss the problem with him”), and “When my child behaves well, it is important to set up rewards or privileges”). Cronbach’s alphas were .75, .76, and .70, respectively, at pretest, posttest, and follow-up.

Observed externalizing behavior

The Dyadic Parent–Child Interaction Coding System (i.e., DPICS) was used to measure observed externalizing behavior (Robinson & Eyberg, 1981, Webster-Stratton, 1989). At all three measurement times, parent-child dyads were observed for 20 minutes, divided into four five-minute episodes: (1) free play (i.e., to get used to being video-taped), (2) child-directed play (i.e., child picked a toy and directed the session) (3) parent-directed play (i.e., parent picked a toy and directed the session), and (4) clean-up (i.e., parent instructed child to clean-up). In the latter three episodes, negative child behavior was coded using 5 categories: indirect command non-compliance, direct command non-compliance, cry-whine-yell, destructive behavior, and physical negative behavior. A total summed score based on these three episodes and reflecting the frequency of these behaviors served as the observational index of externalizing behavior. The inter-episodes correlations among within-episodes composites were significantly correlated at pretest (r = .11-.48), posttest (r = .25-.40), and follow-up (r = .17-.38) measurement occasions. Observations were coded by six trained research assistants blind to condition and measurement wave. Monthly calibration meetings were held to prevent observer drift. A random 20% of observations were independently coded by two coders unaware which observations would be used to assess inter-observer agreement. Interrater reliability, based on intraclass correlation (ICC), was .83, .82, and .70, respectively, at pretest, posttest, and follow-up.

Parent-reported positive behavior

The Parent Practices Inventory (PPI: Webster-Stratton, 2001a) used at all measurement occasions assessed parenting skills and discipline styles. Although positive parenting behavior was also observed, the fact that little variance was detected in the observational data precluded us from using this measure as an outcome. The PPI consists of 15 scales, each containing several items related to how parents typically respond to their child’s appropriate and inappropriate behavior (measured using a 7-point Likert-type scale: 0 = not likely at all/never to 7 = very likely/always). For this report, praise and incentives (11 items) and positive verbal discipline (9 items) were combined to create an index of positive parenting behavior (e.g., “When my child complete chores I praise him,” “When my child refuses do to something I discuss the problem with him), and “When my child behaves well, it is important to set up rewards or privileges”). Cronbach’s alphas were .73, .76, and .79, respectively, at pretest, posttest, and follow-up.

Genotyping

Genotyping was conducted at BaseClear laboratories, Leiden, The Netherlands, using well-established methods. Buccal swabs collected from children were incubated in lysis buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/mL protease K, and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples.
using the Chemagene buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). All analyses were performed automatically using specialized genotyping software. Each plate’s results were checked by a lab-worker (and checked by a second worker), those showing notable deviations or failings were repeated. As a control check, each 96-well plate contained one blank and analyses were continued only if the blank showed a negative result. Overall, genotyping yielded a success rate of approximately 99% or higher for all polymorphisms central to this report.

**DRD2 and COMT**

To determine the SNP’s of the DRD2 rs1800497 and COMT rs4680 polymorphisms, 1 ul of the isolated samples were analyzed using TaqMan® chemistry (Cat. # 4351575, Applied Biosystems). Samples were run on an ABI-7500 Real-Time PCR instrument and data were analyzed using 7500 System SDS software. DRD2 genotypes \( n = 247 \) A2/A2, \( n = 122 \) A2/A1, \( n = 14 \) A1/A1 were in Hardy–Weinberg equilibrium (HWE), \( \chi^2 (1, n = 383) = 0.5, p = .82 \) \( n \) no genotyping), COMT genotypes \( n = 90 \) met/met, \( n = 185 \) met/val, \( n = 108 \) val/val) were in HWE, \( \chi^2 (1, n = 383) = 0.4, p = .84 \) \( n \) no genotyping).

**DAT1**

For all VNTR polymorphisms (i.e., DRD4, DAT1, and MAOA) one microliter of PCR product was mixed with 0.3 ml LIIZ-500 size standard (Applied Biosystems) and 11.7 ml formamide (Applied Biosystems) and run on a AB 3730 genetic analyser set up for fragment analyses with 50 cm capillaries. Results were analysed using GeneMarker software (Softgenetics). The region of interest from the following cycling conditions: initial denaturation step of 10 min at 95°C, followed by 27 cycles of 30 sec 95°C, 30 sec 60°C, 60 sec 65°C and a final extension step of 10 min 65°C. Genotypes \( n = 248 \) no7-repeat/no7-repeat, \( n = 119 \) no7-repeat/7-repeat, \( n = 8 \) 7-repeat/7-repeat) were in HWE, \( \chi^2 (1, n = 375) = 2.11, p = .15 \) \( n = 12 \) no genotyping).

**MAOA**

The region of interest from the MAOA gene was amplified by PCR using the FAM-labelled MR primer \( 5’-GGATACACCTCAGCAGG-3’ \), forward primer \( 5’-ggatatcaacttcacgagcagcggagcaagcag-3’ \) and a reverse primer \( 5’-GGACTGCTGGCCACCTGGTC-3’ \). Typical PCR reactions contained between 10 and 100 ng genomic DNA templates, 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 DNA Polymerase (NEB) in a total volume of 30 ml 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. Genotypes for boys were \( n = 80 \) low/low and \( n = 123 \) high/high \((n = 11 \) no genotyping). Since boys have only one X chromosome, only girls were included in the HWE calculation. Genotypes for girls \( n = 23 \) low/low, \( n = 75 \) low/high, \( n = 96 \) high/high were in HWE, \( \chi^2 (1, n = 167) = 0.13, p = .73 \) \( n = 6 \) no genotyping).

**Polygenic scoring**

Each polymorphism was assigned a point if the child was carrying at least one of the putative plasticity alleles; these values were then summed to create a polygenetic plasticity index ranging from 0-5. To create groups of reasonable size for statistical analysis, children scoring low (0-2) and high (3-5) on this polygenic index were distinguished. The distribution of those scoring low or high on the polygenic index was, for boys, respectively, 23.2% \((n = 44) \) and 29.5% \((n = 56) \) in the control group and 17.9% \((n = 34) \) and 29.5% \((n = 56) \) in the intervention group. The comparable distribution for girls was, respectively, 19.9% \((n = 23 \) no genotyping) and 29.5% \((n = 30) \) in the control group and 39.7% \((n = 60) \) in the intervention group and 12.6% \((n = 19) \) and 27.8% \((n = 42) \) in the intervention group.

**RESULTS**

Latent growth curve modeling (LGCM) in Mplus (Muthén & Muthén, 2008-2015) was performed initially on all children, irrespective of their race/ethnicity and separately for boys and girls, to assess the development of externalizing behavior across pretest, posttest, and follow-up assessments. Because individual growth is estimated for each child, LGCM is an excellent approach for examining variation in the development of externalizing behavior while considering whether certain predictors are associated with differential trajectories. Full information maximum likelihood (FIML) was used to treat missing data. Experimental condition (i.e., intervention vs. control) and...
the polygenetic plasticity index (i.e., more vs. few) served as predictors in the primary model. Because the intervention was focused on inducing positive parenting behavior, and presumed to affect children by changing parenting, we used a Parallel Process LGM to evaluate whether genetically moderated intervention effects were more pronounced when parents increased more rather than less in positive parenting behavior (i.e., gene × slope positive parenting on slope child externalizing behavior) (Cheong, MacKinnon, & Khoo, 2003). Model fit is considered good if the Root Mean Square Error of Approximation (RMSEA) is <.08 and mediocre if >.10. Confirmatory Fit Index (CFI) values should be >.95 (Hu & Bentler, 1999).

**Genetic, intervention, and gene × intervention effects**

Parent-reported child externalizing behavior

With regard to specific effects, we consider first effects on problem behavior at pretest before turning attention to change over time (i.e., slope). Inspection of Table 2 indicates that for both boys and girls there were no significant main effects of treatment condition (i.e., IY vs. control) on the pretest intercept (boys: $B_0 = -0.36, p = .65$; girls: $B_0 = -0.01, p = .99$), thereby indicating that the randomization process proved effective in equating groups for initial levels of problem behavior. For girls there was a significant main effect of the polygenic index of plasticity on the pretest intercept ($B_0 = -163, p = .03$), indicating that girls who scored high on the index had fewer problems initially. Turning to the prediction of slope, treatment condition proved significant in the case of both boys ($B_1 = -0.41, p = .04$) and girls ($B_1 = -0.055, p = .05$), revealing that parent-reported externalizing behavior decreased more in the intervention than control group. The models of intercept and slope showed a relatively good fit for boys ($\chi^2 (df = 3, n = 190) = 6.70, CFI = .99, RMSEA = .08$) but a mediocre fit for girls ($\chi^2 (df = 3, n = 151) = 6.45, CFI = .98, RMSEA = .09$).

In a second phase of modeling the condition × polygenic index interaction term was included. This two-way interaction proved significant for slope for boys ($B_0 = -183, p = .01$) ($\chi^2 (df = 4, n = 190) = 2.21, CFI = 1.00, RMSEA < .001, \eta^2 = .04$), though not for girls ($B_0 = 0.08, p = .37$) ($\chi^2 (df = 4, n = 151) = 7.06, CFI = .98, RMSEA = .07, \eta^2 = .00$). Thus, the intervention was most effective in decreasing parent-reported externalizing behavior for boys with high polygenic scores, particularly by time of follow-up (partial $\eta^2 = .20$) (see Figure 1). A series of planned comparisons testing the hypothesis that the high-polygenic-index boys in the intervention group would benefit most from the intervention, revealed that in comparison to all other boys, this hypothesized highly-susceptible subgroup of children assigned to the intervention (1) did not differ from all other boys on parent-reported externalizing behavior at pretest ($F(1, 180) = 1.00, p = .40$), but (2) scored significantly lower at follow-up ($F(1, 180) = 3.78, p = .01$) and thus (3) evinced significantly greater reduction (i.e., change) from pretest to follow-up than all other boys ($F(1, 180) = 5.29, p = .01$).

**Observed child externalizing behavior**

Inspection of Table 3 indicates that the main effect of condition on the pretest intercept of observed externalizing behavior proved significant ($B_0 = 196, p = .01$), as did that for slope ($B_1 = -108, p = .03$), but only for boys ($\chi^2 (df = 5, n = 188) = 4.92, CFI = 1.00, RMSEA = .02, \eta^2 = .05$), not girls (intercept: $B_0 = 0.20, p = .81$; slope: $B_1 = -0.28, p = .56$) ($\chi^2 (df = 3, n = 151) = .001, CFI = 1.00, RMSEA = .001, \eta^2 = .001$).
Gene × Positive-parenting change

As a preliminary step before evaluating whether the G × I effect would prove most pronounced when parents evidenced the most increase in positive parenting, we evaluated whether, in general, parents assigned to the experimental group increased more in positive parenting than those assigned to the control group. This expectation was confirmed. Although condition proved non-significant for pretest intercept of reported positive parenting behavior (\( B = 1.31, p = .53 \)), it was significant for slope (\( B = 1.41, p = .001 \)) \( \chi^2 (df = 12, n = 190) = 52.42, CFI = .96, RMSEA = .10 \). Thus, compared to the control group, IY parents showed more improvement in positive parenting behavior over time.

To examine whether the effect of this change in positive parenting on change in boys’ externalizing behavior was moderated by the polygenic dopaminergic index, we used the continuous parenting slope variable reflecting change over time in positive parenting behavior to formulate a two-way interaction term involving it and the polygenic index. This two-way interaction proved significant in predicting change (i.e., slope) in reported externalizing behavior (\( B = -.881, p = .04 \)). To graphically depict this interaction, we created two control subgroups (high and low polygenic index) and four intervention subgroups, the latter reflecting whether boys had a high or low polygenic score combined with whether their parent increased a lot or a little in positive parenting behavior: low polygenic – low positive parenting increase (n = 14), low polygenic – high positive parenting increase (n = 18), high polygenic – low positive parenting increase (n = 20), and high polygenic – high positive parenting increase (n = 38). Inspection of Figure 2 reveals that boys with high scores on the polygenic plasticity index whose parents increased most in positive parenting evinced the greatest decline in parent-reported externalizing behavior; note, though, that the individual slope for boys scoring high on the polygenic index with positive parenting behavior remained positive.

Sensitivity analyses

As a robustness check, a final series of analyses were undertaken (see Table 4). These sought to determine whether the significant results reported pertaining to the genetic moderation of intervention efficacy in the case of boys would hold under

OO). Compared to the control group, IY boys showed more externalizing behavior at pretest, but also the steepest decrease over time when controlling for baseline differences. Both the main effect of the polygenic index and the condition × polygenic index interaction term proved non-significant for both boys and girls: boys: \( B = -.003, \chi^2 (df = 6, n = 188) = 4.92, CFI = 1.00, RMSEA = .000, \) partial \( r^2 = .00 \); girls: \( B = -.101, \chi^2 (df = 4, n = 151) = 112, CFI = 1.00, RMSEA < .001, \) partial \( r^2 = .00 \) (see Table 5). These latter results indicate that children’s genetic make-up neither predicted their observed externalizing behavior nor moderated the effect of the intervention on this behavior.

The complementary Figures for observed externalizing behavior in boys and parent-reported and observed externalizing behavior in girls are shown in Supplementary Material.

Figure 1. Development of parent-reported externalizing behavior (as indicated by scores on the Eyberg Child Behavior Inventory [ECBI] Intensity Score scale) across pretest, posttest, and follow-up for control and intervention group with 0–2 putative polygenic plasticity alleles or 3–5 such alleles.

Figure 2. Development of parent-reported externalizing behavior (as indicated by scores on the Eyberg Child Behavior Inventory [ECBI] Intensity Score scale) across pretest, posttest, and follow-up for intervention group with low or high increase in positive parenting (as indicated by slope scores on the construct of Positive Parenting derived from the Parent Practices Inventory [PPI]) and boys with 0–2 putative polygenic plasticity alleles or 3–5 such alleles.

As a preliminary step before evaluating whether the G × I effect would prove most...
varying sampling and design conditions, appreciating that (1) inclusion of children who vary in their race/ethnicity could be problematic when studying G × E (Praprer et al., 2007) and (2) results may differ when only analyzing complete (i.e., completer only) vs. intention-to-treat data. The previously documented G × I effect on the slope of reported externalizing behavior proved significant in all sensitivity analyses: Caucasian boys only with complete data (β = −1.49, p = .05) (χ² [df = 4, n = 168] = 2.92, CFI = 1.00, RMSEA = .00); all boys with intention-to-treat data (β = −1.84, p = .01) (χ² [df = 4, n = 210] = 4.92, CFI = 1.00, RMSEA = .05); and Caucasian boys only with intention-to-treat data (β = −1.68, p = .02) (χ² [df = 4, n = 185] = 6.13, CFI = 99, RMSEA = .05). Similarly, as the data displayed in Table 4 indicate, boys with high polygenic scores whose parents increased a lot in the intervention condition showed the greatest decline in parent-reported externalizing behavior in all analyses (though in one case the effect was not significant, p < .08). These results underscore the robustness of results in the primary analyses chronicling the genetic moderation of intervention efficacy.

Results revealed that boys, but not girls, carrying many putative plasticity alleles decreased significantly in parent-reported, but not observed externalizing behavior as a result of their parents’ involvement in the IY program. Such IY-treatment-induced change was not evident in boys in the experimental group carrying few dopaminergic plasticity alleles—or boys assigned to the control group, irrespective of the latter’s polygenic plasticity score. These results are consistent with the meta-analytic findings of Van IJzendoorn and Bakermans-Kranenburg (2015) indicating that effects of diverse experimental manipulations and interventions are substantially stronger in the case of carriers of putative plasticity alleles than those presumed, for genetic reasons, to be less susceptible to environmental influences. Upon first consideration, the findings for boys appear consistent with the differential susceptibility theory (Belsky et al., 2007, Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005). The fact, however, that children assigned to the control group, who had many plasticity alleles did not evince the greatest increase (or least decrease) in problems over time means that the for-worse pattern of change did not materialize, only the for-better pattern.

Especially notable with respect to the G × I findings is that the genetically moderated intervention effect (on boys’ parent-reported externalizing behavior) proved most pronounced when positive parenting behavior improved the most in response to the IY program. This seems to validate the claim that parent training effects, like IY, on children’s problem behavior are indirect and due to effects on positive parenting behavior (see also, Klein Velderman et al., 2006). Such results raise questions about why some parents changed more than others—in a positive way—in response to the intervention. Quite conceivably it could have something to do with their own genetic make-up and therefore their dopaminergic plasticity. Unfortunately, this critical issue could not be addressed herein because genetic data on parents were not available (see Chhangur et al., 2015).

It is also notable that evidence of genetic moderation of intervention efficacy only emerged in child behavior reported by parents and not in case of observed externalizing behavior. This is consistent with the overall intervention effect for this sample.
Genetic Moderation of Intervention Efficacy: Dopaminergic Genes, The Incredible Years, and Externalizing Behavior in Children

Figure 1a. Development of observed externalizing behavior in boys (as indicated by scores on the Dyadic Parent–child Interaction Coding System [DPICS] Child Negative Behavior Scale) across pretest, posttest, and follow-up for control and intervention group with 0–2 putative polygenetic plasticity alleles or 3–5 such alleles.

(see Weeland, Chhangur et al., in press). It seems plausible that the null G × I findings reported here for observed behavior could be due to the limited sampling period (i.e., 20 months) and highly structured observation context. After all, parental reports reflect, presumably, extensive opportunity to observe child behavior across days, weeks, and months during situations.

Although the overall effects of the IY parent program (on boys and girls together) were more pronounced at the immediate posttest relative to the delayed follow-up (see Weeland, Chhangur et al., in press), inspection of Figures 1 and 2 makes clear that in the case of the more genetically susceptible boys, treatment effects were not especially evident immediately after the intervention, but became so by about four months later. This observation suggests that it may take time, in the case of more genetically susceptible boys, for increases in positive parenting induced by the IY intervention to become consolidated and thus influence child behavior. Parenting interventions are designed to change the well-established, coercive and repetitive cycle of aversive parent–child interactions that induces and maintains antisocial behavior (coercive interaction, Patterson, 1982). Thus, it may take a while before increases in parenting-based reinforcement processes become established as a result of the IY program, thereby down-regulating externalizing behavior.

Although the boys scoring lower on the polygenic index in the experimental group changed less than those with higher polygenic scores, the question arises whether this would have proven to be the case had the intervention lasted longer. Had more time been allowed for parents to improve their parenting, via more intervention sessions and/or as a result of a later occurring follow-up evaluation, it is possible that the intervention children with fewer plasticity alleles could have caught up with those who responded more quickly to the IY intervention. Also, a very different intervention—or even one administered at an earlier age—might have proven more effective with children with fewer plasticity alleles. Thus, even if we get to the point where we could confidently conclude that a particular program—like IY—does not work equally well for all, this would not lead to the conclusion that the unaffected children are entirely non-susceptible to intervention effects, but rather that different children may benefit from different approaches (e.g., Chorpita & Daleiden, 2009).

It remains unclear why the hypothesized G × I effects emerged only in the case of boys. Importantly, this was not a statistical artifact of there being greater variance in boys’ than girls’ externalizing behavior. In fact, the overall main effect of the intervention was similar in both subsamples in case of reported externalizing behavior. Nevertheless, we are not the first to document polygenic moderation of an environmental effect that is restricted to boys. Belsky and Beaver (2011) observed the same when investigating effects of parenting on adolescent self-regulation in their non-experimental research. Such results led them to speculate that girls may be more easily socialized, which could account for why girls carrying fewer putative plasticity alleles proved as subject to the parenting effects as those carrying many.

It will take additional research to determine whether the Belsky and Beaver (2011) proposal indeed explains the variation in G × I findings across boys and girls chronicled herein. Further work is also called for to gain insight into the processes that could explain how the individual genes included in our polygenic index influence
dopaminergic functioning in the brain and, thus, make some boys seemingly more susceptible to the beneficial effects of IY than others.

In addition to raising intriguing issues for future research, the current inquiry had multiple strengths. Most notably, it involved an experimental research design and focused on multiple rather than single candidate genes known to play a role in the functioning of dopaminergic neurotransmitter system. Genes selected were based on prior differential-susceptibility-related \( G \times E \) and \( G \times I \) findings. These strengths do not obviate limitations that must be acknowledged. Perhaps the most important, which applies to almost all RCTs, is that the generalization of results might be limited to families willing to be randomized, with equal chance of being assigned to an experimental and control group. Whereas some of the families that did not enroll may have been put off by the demand of attending 14 weekly sessions lasting two hours, others may have not been willing to take the chance of receiving no intervention service. Not to be overlooked is the relatively modest sample size. Even though an experimental design increases power relative to an observational study (Bakermans-Kranenburg & Van Ijzendoorn, 2015), especially when testing a moderated intervention effect, it is certainly possible that a larger sample might have revealed significant intervention effects even in girls or boys carrying fewer putative plasticity alleles.

SUPPLEMENTARY MATERIAL

Table 1a and Table 1b, see page 111 and 112.
GENETIC MODERATION OF INTERVENTION EFFICACY: DISTINGUISHING RECEPTOR-, TRANSPORTER-, AND ENZYME-RELATED DOPAMINERGIC GENES
CHAPTER 7
GENETIC MODERATION OF INTERVENTION EFFICACY: DISTINGUISHING RECEPTOR-, TRANSPORTER-, AND ENZYME-RELATED DOPAMINERGIC GENES

KEYWORDS
- Externalizing behavior
- Gene-by-environment interaction
- Dopaminergic subsystems
- Incredible Years

PRESS
- Chhangur, R. R., Weeland, J., Overbeek, G., Van der Giessen, D., Matthys, W., Orobio De Castro, B., & Belsky, J. Genetic moderation of intervention efficacy: Distinguishing receptor-, transporter-, and enzyme-related dopaminergic genes. Manuscript under revision.

ABSTRACT
In this report, we build on results of a prior report documenting the genetic moderation of intervention efficacy in the case of boys scoring high on externalizing problems (Chhangur et al., in press). Because prior results revealed that a polygenic index comprised of five dopaminergic genes (DRD4, DRD2, DAT1, MAOA, and COMT) moderated the effect of the Incredible Years (IY) intervention program, we sought to gain insight into which of three dopaminergic subsystems—receptor, transport and/or enzyme—might be principally responsible for the moderational effect discerned. The randomized-controlled-trial involved 190 families with 4-8-year-old boys who had relatively high levels of behavior problems at screening. Results of latent growth modelling in Mplus showed that the originally detected polygenic moderation effect was specifically caused by the subset of enzyme genes (MAOA, COMT). Boys carrying more enzyme-related plasticity alleles (but not boys carrying fewer of such alleles) decreased significantly in externalizing behavior as a result of their parents’ involvement in IY intervention program. This effect remained even when the other dopaminergic genes were controlled for.

Externalizing behavior problems evident early in life tend to persist if not treated, which stresses the importance of prevention efforts (Campbell et al., 2006, 2000). The etiology of early externalizing problems includes diverse environmental factors among which especially problematic parenting have been shown to play a crucial role (Miner & Clarke-Stewart, 2008; Patterson, 1976). Intervention efforts aimed at reducing externalizing behavior therefore targets parenting (e.g., McCart et al., 2006). A meta-analysis of the effectiveness of one such parenting intervention, the Incredible Years program (IY; Webster-Stratton, 2001), revealed it to be effective in preventing and remediating externalizing problems (Menting et al., 2013). However, given the modest effect size of IY, especially in the context of indicated prevention (i.e., Cohen’s $d = 20$), there might be substantial heterogeneity in program efficacy. Because this is true for most child-related interventions, be they preventive, curative or enhancement oriented, insight into the determinants of such heterogeneity might be crucial for boosting effectiveness of these programs (Bakermans-Kranenburg & Van IJzendoorn, 2015; Belsky & Van IJzendoorn, 2015).

Recent research indicates that children’s genetic make-up plays a significant role in predicting which children benefit most and least from a variety of interventions (e.g., Brett et al., 2015; Brody, Beach, Philibert, Chen, & Muny, 2009; Plak, Kegel, & Bus, 2015), including the IY program (Chhangur et al., in press). Specifically, a recent meta-analysis showed that there is repeated evidence of such “for-better-and-for-worse” environmental effects in research on gene-by-environment ($G \times E$) interactions (Van IJzendoorn & Bakermans-Kranenburg, 2011). This may not come entirely unexpected as differential-susceptibility theorizing stipulates that the very personal factors associated with children’s vulnerability to adversity, may also be associated with these children benefiting more than others from environmental support and enrichment (Belsky et al., 2007; Belsky & Pluess, 2009, 2015; Boyce & Ellis, 2005). But most notable for purposes of this report is a meta-analysis showing that a variety of dopamine-related polymorphisms moderate a variety of environmental effects in a differential-susceptibility-related manner (Bakermans-Kranenburg & Van IJzendoorn, 2011).

When it comes to evidence of genetic moderation of environmental effects, studies of gene-by-intervention ($G \times I$) interactions—including random assignment of participants to treatment conditions—have several advantages over correlational $G \times E$ investigations (Bakermans-Kranenburg & Van IJzendoorn, 2015; Belsky & Van IJzendoorn, 2015). One of the most well-acknowledged limitations of non-experimental $G \times E$ research is that a gene-environment correlation ($r_{GE}$) can “masquerade” as putative $G \times E$ interactions (Bakermans-Kranenburg & Van IJzendoorn, 2015). Even when a particular $G$ in a longitudinal $G \times E$ design proves unrelated to the E in question, other unmeasured $G$’s could be influencing the environmental factor under examination and thus confound the interpretation of any detected $G \times E$. Consider in this regard the results of one $G \times E$ study which found that a significant $G \times E$ effect was no longer significant after controlling for parents’ genes (Chhangur, Overbeek, et al., 2015).
It is notable that a variety of very recent \(G \times I\) studies document the moderational role of dopaminergic polymorphisms in conditioning experimentally induced environmental effects (Belsky & Van Ijzendoorn, 2015; Van Ijzendoorn & Bakermans-Kranenburg, 2015; Chhangur et al., in press). Consider in this regard work by Van den Hoofdakker and colleagues (2012) showing that children carrying 10-repeat alleles of the DAT1 benefited most from a behavioral parenting intervention designed to treat children with ADHD. Consider, too, research by Brody and associates (2015) demonstrating that a family-based intervention designed to prevent substance use proved effective principally in teenagers that carried the same plasticity allele.

To date, virtually all such \(G \times I\) studies have focused on the moderating role of single candidate genes (but see Musci et al., 2015; Thibodeau, Cicchetti, & Rogosch, 2015 as exceptions). It is widely appreciated, however, that multiple genes play a role in child development more generally.

System-level genetic approach: Receptor, transporter, enzyme

Dopamine is an excitatory neurotransmitter involved in motivational, attentional, and reward processes (Padmanabhan & Luna, 2014). A complex balance between the amount of dopamine synthesized, released, stored, recaptured, and metabolized determines the intensity of dopaminergic signaling (Bonisch & Eiden, 1997; Roth & Elsworth, 1995). The five aforementioned genes play such roles in dopamine availability in the synapse and thus neuro signaling by either affecting the amount of dopamine (1) released (via neural signaling), (2) recaptured or (3) degraded (i.e., metabolized), possibly making children more and less prone to, for example, environmental cues of reward (Matthys et al., 2013; Moore & Depue, 2016).

\(DRD2\) and \(DRD4\) are considered receptor genes that modulate dopaminergic neural signaling and dopamine release in the synaptic cleft. In the normal process, dopamine is released by a presynaptic neuron into the synapse, where it binds to autoreceptors on the postsynaptic neuron, sending a signal to that neuron. Dopamine is then released for the autoreceptors to be recaptured by transporter proteins or metabolized by enzymes. Thus, these two genes modulate dopamine receptor availability in the brain and, thereby, dopamine signaling and release (Forbes et al., 2009; Noble et al., 1997). The A1 allele of \(DRD2\) and the 7-repeat of \(DRD4\) are treated as plasticity alleles herein (e.g., Bakermans-Kranenburg et al., 2008; Belsky & Beaver, 2011; Chhangur et al., in press, Mills-Koonce et al., 2007) and are functionally associated with lower receptor density and thus with less dopamine signaling/release (Forbes et al., 2009).

\(DAT1\) is considered a transporter gene that modulates the recapturing of dopamine from the synaptic cleft. Along the presynaptic neuron are transporter proteins which transport the dopamine released by autoreceptors back into the neuron, terminating the dopamine signal. In the normal process, transporters recapture dopamine in the synaptic cleft to terminate ongoing signaling, thereby enabling new dopaminergic neural signals. Thus, this gene modulates dopamine transporter availability and, thereby, the recapturing of dopamine used for new neuro signaling and ending ongoing dopamine signals (e.g., Heinz et al., 2000; Mill, Asherson, Brown, D’Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005). Too much transporter availability might lead to too much or too fast recapturing, thereby ending the dopamine signaling too quickly. The 10-repeat allele of the \(DAT1\) is treated as plasticity allele herein (e.g., Belsky & Beaver, 2011; Chhangur et al., in press, Laucht et al., 2007) and is functionally associated with higher transporter density and thus with higher/faster recapturing, that in turn might result in less (ongoing) dopamine signaling (Feltin, Montag, Markett, Walter, & Reuter, 2011; Mill et al., 2002; Yang et al., 2007). However, other studies found the 10-repeat allele relative to the 9-repeat allele to be associated with lower transporter availability and thus less recapturing, thereby increasing the duration of dopamine available at postsynaptic autoreceptors and dopamine signaling (e.g., Van de Giessen et al., 2009).

\(MAOA\) and \(COMT\) are considered enzyme-related genes that degrade dopamine availability in the brain. The enzymes are present in both the presynaptic neuron and synaptic cleft. In the presynaptic neuron the enzymes break down the dopamine that has been transported here, while the enzymes in the synaptic cleft...
break down the remaining dopamine in the synapse. Thus, these two genes affect dopamine enzyme availability and, thereby, the degradation of dopamine in the brain (Boulton & Eisenhofer, 1997, Chen et al., 2004). The low-activity allele of the MAOA and the val allele of the COMT are treated as plasticity alleles herein (e.g., Belsky & Beaver, 2011; Chhangur et al., in press; Van IJzendoorn et al., 2008). The val (or high-activity) allele of the COMT is functionally associated with higher enzyme density and greater degradation and thus might result in less dopamine signaling (Lachman et al., 1996). However contradictory, the precise role of the low-activity MAOA allele is unclear, as it presumably functionally associated with lower enzyme density and thus with lower degradation; this would be expected to result in more dopamine signaling (Boulton & Eisenhofer, 1997). Some research on the functional effects of the MAOA, however, fails to document significant associations between the low-activity allele and lower enzyme density in the human brain, even if such was discerned when a related haplotype was the focus of inquiry (e.g., Balciuniene, Emilsson, Orelend, Pettersson, & Jazin, 2002). More research is needed to illuminate the precise role of the MAOA low-activity allele, especially as this allele has been associated with developmental plasticity (e.g., Belsky & Beaver, 2011; Belsky & Pluess, 2009; Kim-Cohen et al., 2006). Despite the fact that COMT and MAOA plasticity alleles are suspected of having opposite effects on dopamine signaling, they also have effects on multiple neurotransmitters (Illi et al., 2003; Matthys, Vanderschuren, & Schutter, 2013) and this may why both contribute to greater responsiveness to environmental conditions. To be clear, all other plasticity alleles considered herein have been related to less dopamine signaling (e.g., Bakermans-Kranenburg & Van IJzendoorn, 2011).

Dopamine modulates the value of rewarding experiences, thereby implying that dopamine signaling is needed to experience rewarding effects (e.g., Pessiglione et al., 2006; Schultz, 2010). It has been appreciated that, in particular, less dopamine signaling may result in: reduced salience of rewarding experiences (Buckholtz et al., 2010); reduced valence of specifically delayed rewarding experiences (Comings & Blum, 2000); reduced arousal in response to rewards (Schultz, 2002); and reduced effects of reward-based learning (Comings & Blum, 2000). As such, it might very well be that children with less dopamine signaling are possibly slower in their reward-based learning and, thus, need more immediate and salient rewarding feedback to increase rewarding experiences, reward-based learning, and physiological arousal (Matthys et al., 2012). Therefore, they may be more sensitive/responsive to immediate positive reward than children high on dopamine (Weeland et al., 2015). Since intervention efforts, like IV, are designed to foster skilled parenting by, in part, promoting immediate positive reinforcement to positive child behavior (i.e., reward and praise), children with less dopamine signaling may disproportionately benefit from positive change in parenting behavior, leading to decreases in externalizing behavior. But again in this regard additional work on neurochemistry is needed to address the exact role of MAOA, hopefully illuminating why it is that children with low-activity allele, presumably associated with more dopamine signaling, are more developmentally responsive to environmental conditions (Byrd & Manuck, 2014; Ficks & Waldman, 2014; Taylor & Kim-Cohen, 2007).

In summary, the functional distinctions just made regarding a set of dopamine genes, based on a system-level approach and developmental plasticity, have not been considered in child developmental research on G x E or G x I interaction, even in investigations that have relied on polygenic indices (Belsky & Beaver, 2011; Chhangur, et al., in press; Musci et al., 2015; Thibodeau et al., 2015). Therefore, we explore the proposition that one or more of the three dopaminergic subsets of plasticity alleles just highlighted—receptor, transporter, enzyme—might be primarily responsible for the polygenic moderation of IV efficacy already detected in the case of boys (Chhangur et al., in press) about the same data set. This approach can shed light on which biological processes might specifically contribute to heterogeneity in program efficacy.

**METHODS**

**Participants**

Data for this report comes from the ORCHIDS study (Observational Randomized Controlled Trial on Childhood Differential Susceptibility) being carried out in The Netherlands (Chhangur, Weeland et al., 2012; Weeland, Chhangur et al., in press). ORCHIDS investigates differential effectiveness of IV intervention program aimed at reducing externalizing behavior in 4- to 8-year-old children. The study consisted of a screening phase in a general population sample and a randomized-controlled-intervention phase in a selected subsample of children with moderate-to-high levels of externalizing behavior. For detailed information about CONSORT schedule and sample descriptive, see Weeland, Chhangur et al., in press). Cheek cells were collected for purposes of DNA assaying from 385 children (failed genotyping assay: n = 2). Building on the report of Chhangur et al., (in press), the current study investigates only boys and their parents (n = 212) with the primary analysis sample consisting of 190 families (excluding intervention dropouts: n = 22).

**Design**

Two months after the screening trained research assistants conducted pretest home visits to assess DNA and parent-child observations. Also, questionnaires (mailed to parents a week earlier) were collected. After the pretest, families were randomly assigned to either the control group (n = 190) or the intervention group (n = 197), with 100 boys in the former and 90 in the latter. Approximately four months (i.e., posttest) and eight months (i.e., follow-up) after the pretest, parents again completed child-behavior questionnaires and parent-child observations were assessed. The Institutional Review Board in The Netherlands (METC UMC Utrecht, protocol number 11-320/K) approved the study.

- Chapter 7
Genetic Moderation of Intervention Efficacy: Distinguishing Receptor-, Transporter-, and Enzyme-Related Dopaminergic Genes

In view of the fact that measures used in the current report have been described in detail in the prior report documenting the polygenic moderation of IY efficacy in the case of boys (Changur et al., in press) on which the current study builds, they are only listed here in the interest of saving space: parent-reported externalizing behavior (ECB; Eyberg & Pincus, 1999) and the five polymorphisms now scored in terms of the subsets to which they belong: receptors (DRD2, DRD4), enzymes (MAOA, COMT), and transporters (DAT1). Following Changur et al., (in press), scoring involved assigning one point whenever a boy carried at least one of the putative plasticity alleles; these values were then summed to create a plasticity index with the receptor and enzyme ranging from 0–2 and transporter 0–1. As in the prior work (Changur et al., in press), we only found a genetic moderation effect on reported, but not observed, externalizing behavior; thus, the latter measure was not used here. The distribution of boys scoring 0, 1, or 2 in the receptor-subset was, respectively, 46.0% (n = 46), 47.0% (n = 47), and 70.0% (n = 7) in the control group and 38.9% (n = 35), 48.9% (n = 44), and 12.2% (n = 11) in the IY intervention group. For the enzyme-subset, respectively, 15.0% (n = 15), 58.0% (n = 58), and 27.0% (n = 27) in the control group and 15.6% (n = 14), 55.6% (n = 50), and 28.9% (n = 26) in the intervention group. Finally, for the transporter-subset, respectively, 90.0% (n = 9) and 91.0% (n = 9) in the control group and 6.7% (n = 6) and 93.3% (n = 84) in the intervention group. Chi-square statistics showed no significant differences in distribution across groups.

RESULTS

Latent growth curve modeling (LGM) in Mplus (Muthén & Muthén, 2008-2015) was performed to assess the development of externalizing behavior for males at the time of pretest, immediate-posttest (following four months of intervention) and follow-up (four months later). In three separate analyses, one for each dopamine-polymorphism subset (i.e., receptor, transporter, enzyme), a first model tested main effects of condition and genotype and a second the interaction of condition and genotype (with main effects included). In each set of analyses, the main effect of condition proved significant, but not the main effect of genotype (see Table 1). More importantly, the G × I interaction also proved significant for slope in the case of enzymes (B1 = -.348, p = .006), though not for either the receptor subset (B1 = .035, p = .53) (χ² [df = 4, n = 190] = 13.02, CFI = .96, RMSEA = .11) or the transporter subset (B1 = -.015, p = .91) (χ² [df = 4, n = 190] = 13.02, CFI = .96, RMSEA = .11).

In the second robustness check, we repeated the LGM analysis which originally included all genes in the polygenic index, but this time eliminating one subset of genes at a time from the index (i.e., first transporter and then receptor genes). This allowed us to examine changes to model fit or parameter estimates upon comparing the original polygenic index and these revised versions. The overall interaction between experimental condition (i.e., intervention group, in comparison to all other boys, (1) did not differ on externalizing behavior at pretest (F(1, 184) = 91, p = .035), but (2) scored significantly lower at follow-up (F(1, 178) = 4.58, p = .03) and thus (3) evinced significantly greater reduction from pretest to follow-up (F(1, 178) = 12.95, p < .001).

Two secondary analyses were undertaken to evaluate the robustness of the results just reported. In a first robustness check, the analysis was re-run including, as covariates, the other two gene subsets and the interaction of each of their component genes with treatment condition. This enabled us to evaluate the unique moderational effect of dopaminergic enzyme-related genes. Yet again the two-way interaction between the enzyme subset and condition proved significant for slope (B1 = -.166, p = .03) (χ² [df = 8, N = 190] = 15.01, CFI = .97, RMSEA = .07).

In the second robustness check, we repeated the LGM analysis which originally included all genes in the polygenic index, but this time eliminating one subset of genes at a time from the index (i.e., first transporter and then receptor genes). This allowed us to examine changes to model fit or parameter estimates upon comparing the original polygenic index and these revised versions. The overall interaction between experimental condition (i.e., intervention group

| Table 1. G × E interactions Between Condition and Decomposed Dopaminergic Subsystems in Development of Reported Externalizing Behavior |
| --- | --- | --- | --- |
| Predictor | Condition | Intercept | Slope |
| Control | -0.04 (0.21) | 0.02 (0.02) | 13.02 (4) |
| Intervention | -0.05 (0.21) | 0.03 (0.03) | 13.02 (4) |
| | | | .96 (4) |
| | | | .97 (4) |
| Enzymes | | | .07 (4) |
| Receptors | | | .11 (4) |
| Transporters | | | .11 (4) |

Note. df = degree of freedom; CFI = comparative fit index; RMSEA = root mean square error of approximation; Condition; 0 = control group; 1 = intervention group. *p < .05.
intervention vs. control) and the original polygenic plasticity index (0–5) proved significant for slope ($B = -0.09, p = .02$) ($t = 4, N = 190$) = 12.78, CFI = 97, RMSEA = 11). After excluding the transporter genes, the interaction again proved significant ($B = -1.05, p = .02$) ($t = 4, N = 190$) = 12.54, CFI = 97, RMSEA = 11). After excluding receptor genes (while retaining transporter genes), the interaction was no longer significant ($B = -0.09, p = .05$) ($t = 4, N = 190$) = 13.22, CFI = 96, RMSEA = 11). These results indicate that even though the model fit did not change significantly, transporter genes nevertheless exerted a less significant influence on the effect of the polygenic index than receptor or enzyme genes.

**DISCUSSION**

In this study, we built on the results of a prior report documenting the moderation of intervention efficacy in the case of boys scoring high on externalizing problems (Chhangur et al., in press). We endeavored to gain insight into which of three dopaminergic subsystems—receptor, transport or enzyme—might be principally responsible for the polygenic moderation effect of the IY effects in the development of externalizing problems and in turn, which biological process contributes to differential responsiveness to the intervention efforts. To our knowledge, no such functional distinctions based on a system-level approach have been pursued to date in either $G \times E$ or $G \times I$ research, including studies using polygenic indices (Belsky & Beaver, 2011; Musci et al., 2015; Thibodeau et al., 2015).

Results revealed that the originally detected polygenic-moderational effect was due to the subset of enzyme genes, and not the subset of genes encoding dopamine receptor or transporter functions. Boys carrying many enzyme-related plasticity alleles decreased significantly more in externalizing behavior as a result of their parents’ involvement in the IY program, compared to boys carrying fewer such alleles. Moreover, this effect remained even when those of the other dopaminergic subsets were controlled. And notably, when adding these subsets as covariates the model fit improved significantly, from being suboptimal (RMSEA = 11) to optimal (RMSEA = 0.7). Important to appreciate, however, is that we conducted three separate tests of $G \times I$. Accordingly, the traditional Bonferroni correction for multiple testing or even the less stringent Benjamini-Hochberg False Discovery Rate correction (i.e. Benjamini & Hochberg, 2005) used in our report on the overall IY effectiveness (see Weeland, Chhangur et al., in press) would have resulted in a nonsignificant effect. This observation highlights the need for replication of the results reported herein.

The $G \times I$ effects detected herein and when using the original polygenic index (Chhangur et al., in press) are consistent with differential susceptibility thinking (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005) in that children with the most enzyme-related plasticity alleles were more sensitive to positive environmental change, induced by IY, with the (untested) presumption being that they would also be more susceptible to the adverse effects of negative contextual conditions (e.g., Belsky & Pluess, 2009; Kim-Cohen et al., 2006). However, as children were screened to have relatively high levels of behavior problems at pretest—presumably indicating an at risk group—we expected the same genetic subgroup that reaped the most benefit from the intervention to also show most externalizing behavior if assigned to the control group (Chhangur, Weeland et al., 2012). This is because environmental risk conditions (i.e., negative parenting) might remain stable or intensify if not treated (Patterson, 1982). This, however, did not prove to be the case.

Enzyme-related dopaminergic genes, relative to receptor- and transporter-related ones, may be especially influential because—apart from their degradation of dopamine—they are also involved in the degradation of norepinephrine and serotonin (Ili et al., 2003; Matthys et al., 2013). Thus, because these polymorphisms impinge on multiple neurotransmitter systems, they may have an especially strong role in children’s susceptibility to environmental signals, and as such may be contributing most directly to the differential-susceptibility effect chronicled in this paper. Ultimately, further work is required to gain insight into specific endophenotypic processes that could explain how the two enzyme genes in question influence dopamine signaling in the brain and, thus, make some boys seemingly more susceptible to the beneficial effects of IY than others. Also, more research is needed to better understand the relation between the functioning of the neurotransmitter systems (e.g., dopaminergic, serotonergic, and noradrenergic) and differential susceptibility processes based on reward and punishment (Matthys et al., 2012). Readers are referred in this regard to a recent paper by Moore and Depue (2016) for a most insightful
description of neurobehavioral foundations of environmental reactivity.

Our initial strategy for decomposing the originally composited dopaminergic genes—each of which had individually been implicated in moderating environmental effects in a differential-susceptibility-related manner—was based on the view that the five genes have different functions that influence dopamine signaling and that it is the additive effect of receptor, transporter, or enzyme that makes some children especially susceptible to the changes in parenting that are presumed to follow from parental participation in the IY program (Nikolova et al., 2011). However, the precise role of the low activity MAOA allele remains unclear as this allele is presumably functionally associated with higher enzyme density and thus more dopamine signaling in the brain (Boulton & Eisenhofer, 1997), while the COMT val allele is functionally associated with lower enzyme density and thus less dopamine signaling (Chen et al., 2004). Thus, although we gained more insight into G × I using a subsystem-level approach based on the functioning of particular dopaminergic genes, more research is needed at an endophenotypic level to illuminate mechanisms of influence. Also, since this is the first study in child-research on G × E that made a distinction between dopaminergic subsets, replication is clearly needed.
GENERAL DISCUSSION
The development of externalizing behavior in children is highly dependent on the quality of parenting they receive (Miner & Clarke-Stewart, 2008; Stormshak et al., 2000), but appears to be explained best by the interaction of genes with environment (G × E) (e.g., Rutter, 2012). However, G × E findings have raised concerns about mixed findings and replications, making it difficult to draw conclusions (Dick et al., 2015; Weeland et al., 2015). One challenge lies in extending correlational G × E studies, by using designs that permit causal inferences and rule out alternative explanations in terms of gene-environment correlations (rGE). Another important challenge lies in understanding how underlying neurobiological processes link genes and environment to child externalizing behavior (Salvatore & Dick, 2015). In this thesis, we sought to clarify G × E interactions in child externalizing behavior based on multiple genes influencing the dopamine system. First, we conducted a longitudinal study that allowed us to predict the development of G × E over time, thereby accounting for passive rGE (chapter 2). Second, an intervention study was carried out in which the environment was experimentally manipulated (chapters 3-7).

**Longitudinal results**

Longitudinal G × E studies permit the investigation of how externalizing behavior unfolds over time and whether parenting behavior predicts change in externalizing behavior in case of a specific genetic subgroup. In chapter 2, we investigated whether the likelihood to develop severe externalizing behavior (delinquency) as a consequence of negative parenting (high psychological control, low parental support), depended in part on genetic variation in two dopamine-related genes (DRD2, DRD4). We found evidence for G × E interactions (DRD2 × parental support, DRD4 × psychological control). The DRD2 interaction was curvilinear, in that adolescents carrying the A2A2 genotype showed steeper increases in delinquent behavior across early to mid-adolescence followed by quicker decreases by late adolescence, in response to low parental support. The DRD4 7-repeat allele interacted with high psychological control – however, when accounting for passive rGE, the interaction was no longer significant, indicating that passive rGE may underlie G × E effects. This needs further investigation in follow-up studies, specifically with experimental studies that rule out alternative explanations for any detected G × E by design.

**Experimental results**

In the following chapters we aimed to resolve concerns about rGE that have plagued longitudinal G × E work, by conducting an experimental intervention study (see chapters 3 and 4). Here, we again considered negative parenting to be a risk factor that contributes to the development of externalizing behavior, but also to be one of the strongest potentially modifiable factors (McCart et al., 2006). We used the behavioral parenting training Incredible Years (IY; Webster-Stratton, 2001) aimed at reducing externalizing behavior in young children. Parents learned to decrease negative child behaviors, by praising, rewarding and coaching the “positive opposite”
of each negative behavior. Thus, IY is focused on reducing negative child behaviors through improving positive parenting strategies and improving parental sensitivity and warmth. However, to show changes in externalizing behavior, children may need to be particularly sensitive to positive parenting strategies, like parental reward and praise. In this study, we tested whether children’s sensitivity to experimentally-induced positive parenting was dependent on genetic characteristics. Because most of G × I (gene-by-intervention) research is limited by the focus on single candidate genes and fails to do justice to the polygenic nature of development, we created a dopaminergic polygenic index including multiple dopaminergic genes. The cumulative consideration of multiple genes, via polygenic indices, might collectively account for significant polygenic effects (Reif & Lesch, 2003). Therefore, rather than focusing on a single dopaminergic polymorphism as a potential moderator, we adopted a systems approach by creating a polygenic index (Nikolova et al., 2011).

We first considered the general effectiveness of the IY program in chapter 5. The main finding was that the intervention significantly improved positive parenting behavior (reported and observed) and that children whose parents received IY showed greater reductions in externalizing behavior (reported but not observed) compared to those in the control group. Then, in chapter 6 we evaluated the genetic moderation of efficacy of the IY program by creating a dopaminergic polygenic index (DRD2 A1, DRD4 7-repeat, DAT1 10-repeat, MAOA low-activity, COMT val allele), based on the prior defined allelic variants in chapter 3 and results listed in chapter 5. As expected, IY was most effective in decreasing reported externalizing behavior in boys carrying more rather than fewer of the dopaminergic polymorphisms. This effect was most evident in boys whose parents showed the strongest increases in positive parenting in response to the intervention. The fact that this identified G × I effect was restricted to reported and not observed externalizing behavior is consistent with the general intervention effect for this sample (chapter 5). Finally, in chapter 7 we built on the results of genetic moderation of intervention efficacy listed in chapter 6, by decomposing the polygenic index in dopaminergic subsystems (receptors, transporters, enzymes). Results showed that the detected polygenic moderation effect was specifically caused by the subset of enzyme-related polymorphisms (MAOA low-activity, COMT val allele).

Different G × E frameworks in explaining sensitivity to environment

Initially, three major patterns of environmental responsiveness have been suggested, that refer to diathesis-stress (e.g., Zuckerman, 1999), differential susceptibility (Belsky et al., 2007, Belsky & Pluess, 2009, 2013, Boyce & Ellis, 2005), and vantage sensitivity (Pluess & Belsky, 2013, Pluess, 2015). In chapter 2 we found evidence for a diathesis-stress type interaction, whereas in chapters 6 and 7 we found evidence for a vantage sensitivity type interaction. More specifically, the results in chapter 2 illustrated that children carrying the DRD2 A2A2 genotype and DRD4 7-repeat polymorphism (before accounting for passive GE) were more vulnerable for negative parenting but did not benefit most from positive parenting. One possible explanation might be that children without these specific genetic variants already scored around zero on delinquent behavior, irrespective of parenting behavior (i.e., floor effect). The results chronicled in chapter 6 and 7 were partly consistent with our a priori defined differential-susceptibility hypotheses in chapter 3, but only supported the for-better pattern of change not the for-worse pattern. Thus, rather than finding evidence for differential susceptibility we found evidence for vantage sensitivity. However, since children were screened to show relatively high levels of externalizing behavior at pretest—presumably indicating an at risk group—we expected children of the same genetic subgroup, who benefited most from the intervention, to also develop most externalizing behavior if assigned to a control group. One possible explanation for not finding differential susceptibility might be that despite our screening procedure we were unable to sample a truly “at risk” group, thereby implying that the environmental risk condition (i.e., negative parenting) did not intensify if not treated (Patterson, 1982). Although this remains speculation, perhaps a differential susceptibility type interaction would have been obtained within a clinical sample that included more severe externalizing behavior and/or more severity in environmental context (e.g., lower social-economic-status families). More specifically, such severity could have triggered a coercive exchange between child and parent (e.g., Patterson, 1982; Scaramella & Leve, 2004), leading to increases in externalizing behavior in the control group. A next step in G × I research could therefore be to oversample high risk families.

Further directions to test differential susceptibility: Nano- and micro-trials

Future research, particularly experimental within-subject designs, will allow more advanced testing of the “for-better-and-for-worse” differential-susceptibility-related hypothesis. The most important proposition of differential susceptibility is that the very same individuals showing the worst outcomes in negative environments would also benefit the most from supportive or enriched environments. Yet we only compared children exposed to either a positive or negative environment using a between-subject design. Nano- and micro-trials in which the same children are exposed to both positive and negative environmental stimuli would be one way of extending the work presented herein and testing hypotheses of differential susceptibility within individuals (Van Uzenna & Bakermans-Kranenburg, 2015). Nano-trials are useful for elucidating immediate behavioral or neural responses to a small range of stimuli. To investigate reward-related differential susceptibility, based on genetic variations, children’s automatic evaluation towards rewarding or unrewarding stimuli could be examined in a within-subject design. Previous research showed that participants reacted more quickly to positive stimuli when instructed to pull a lever toward them (approach-based reaction) and to negative stimuli when instructed to push the lever away (avoidance-based reaction) (Elliott & Covington, 2001). Since approach and avoidance behavior is linked to dopamine release in the
brain (Ledoux, 1995), it would be interesting to examine whether genetic variations in dopaminergic genes moderate these reaction-times to positive and negative stimuli in a differential-susceptibility-related manner. Macro-trials use a manipulation of a broader aspect of the environment. For instance, parents could be assigned to different conditions (enriched vs. negative) in which they are either instructed to add stickers to a chart and provide help (positive rewarding) or to remove stickers and provide no help (negative unrewarding). The child could be instructed to solve puzzles that are quite difficult to complete alone in these different circumstances (e.g., Tangram-puzzle, Wiggly-block). Indeed, less ability to solve problems and less appropriate interpersonal conflict management-skills have been related to early-onset externalizing behavior (Asarnow & Callan, 1985; Maze & Cox, 1990) and could be thoroughly assessed during an experiment (e.g., Webster-Stratton, Reid, & Hammond, 2001). Interestingly, we could test the proposition whether some children are more sensitive to both a positive and negative parenting environment than others based on genetically induced qualities related to the dopaminergic system.

From single genes to a systems approach

In chapter 2, we investigated longitudinal effects of G × E between parenting behavior and the DRD2 and DRD4 genes on delinquent behavior in a subsample of the Family and Health study (Harakeh et al., 2005). However, rather than finding the DRD2 A1 polymorphism to be moderating parenting effects, it was the DRD2 A2 allele that caused changes in externalizing behavior. Indeed, there are mixed findings regarding the DRD2 “risk” variant, especially in case of severe externalizing behavior in adolescence (Eisenberg et al., 2007; Guo et al., 2007; Ratsma et al., 2001; Vasilyev, 2011). Notably, because the results were opposite to what normally would be predicted (e.g., Ratsma et al., 2001), the creation of an index based on a priori expectations would have produced uninterpretable results. However, since several dopaminergic polymorphisms might moderate the association between parenting and child behavior (Bakermans-Kranenburg & Van Uzendoorn, 2011), it has been suggested that complex traits, like externalizing behavior, might be polygenetic in character (Reif & Lesch, 2003). In chapters 6 and 7, we created a polygenic index based on prior differential-susceptibility-related G × E and G × I research findings, particularly found in younger children showing less severe externalizing behavior. The polygenic index based on the dopamine system (chapter 6) and dopamine-subsystem (chapter 7) moderated externalizing behavior in response to intervention-induced positive parenting. These findings indicated that it is likely that complex behavioral traits are indeed influenced by multiple rather than single loci (Nikolova et al., 2011; Reif & Lesch, 2003).

Beyond selecting only single candidate genes, Genome-Wide Environmental Interaction (GWEI) analyses could be used to select (novel) dopamine-related polymorphisms located across the genome on an independent one-by-one basis (Aschard et al., 2012). Polygenetic indices could be created based on significant proven polymorphisms. Also, to reduce the high number of polymorphisms independently tested, generalized multifactor dimensionality reduction software could be used in so-called Genome-Wide Association gene-gene interaction (GWAI) analyses (Kim & Park, 2015). Such an approach would improve statistical power by reducing multiple testing and yet would include multiple theoretically (biologically) related genes based on dopaminergic functioning. However, GWEI and GWAI studies are hypothesis-free and thus it is important to only include hypothesis-driven novel polymorphisms in the polygenic index (e.g., related to less dopaminergic signaling).

Neurobiological foundation of genetic moderation of intervention efficacy

The findings chronicled in this thesis confirmed that the dopaminergic polymorphisms were responsible for genetic moderation, making some children seemingly more sensitive to parenting behavior than others (chapter 2, 6, and 7). However, the specific underlying neurobiological process that mediated the effect of parenting behavior on child externalizing behavior remains unclear. Recently, Moore and Depue (2016) introduced a “threshold model” stipulating that neurobiological sensitivity to environment can be conceptualized as a threshold that is influenced by the function of (1) the level of neurotransmitter activation and (2) the magnitude of environmental stimuli. This theorizing is consistent with our claim that less dopamine signaling might especially result in reduced reward salience and slower reward-based learning, that relate to difficulties in turning attention to or encoding rewarding stimuli (Cummings & Blum, 2000; Schultz, 2002). As such, children with less dopaminergic signaling may have a higher threshold value to be sensitive to reward-based parenting and, importantly, the range of effective rewarding stimuli to prevent/reduce externalizing behavior may be much smaller for parents. More specifically, these children may especially be in need for powerful and immediately positively rewarding stimuli in their environment due to genetically induced qualities of the dopaminergic system, responsible for impaired reward processing (Matthys et al., 2012). Indeed, chapter 6 confirmed that children carrying more dopaminergic polymorphisms benefited most from their parent’s involvement in the intervention program. But most notable, this genetic moderation effect was most pronounced when parents increased a lot in positive parenting, thus implying that their improved parenting quality now fell above rather than below the child’s sensitivity threshold value.

Complexity of differentiating dopaminergic subsets

Even though research on G × E has tended to consider dopamine related genetic variability under a single denominator, in theory different genes might impact different aspects of the dopamine system (Chen et al., 2011). However, in reality distinguishing dopaminergic subsets seems to be complex. For example, both the DRD2 A2 (chapter 2) and DRD2 A1 (chapter 3, 6, and 7) variants are related to increased sensitivity to parenting behavior. In addition, the COMT polymorphism is linked to higher dopamine
degradation and the MAOA polymorphism to lower dopamine degradation (chapter 7). Notably, especially the MAOA is also involved in the degradation of serotonin and norepinephrine. Specifically, higher serotoninergic and norepinephrine functioning is associated with increased sensitivity to environmental context in general (Homberg & Lesch, 2011). Although these results complicate the straightforward interpretation of polymorphisms roles in the dopamine system, this might explain why the MAOA polymorphism—regardless of its contradictory dopaminergic function—relates to increased responsiveness to parenting behavior. That is, the MAOA polymorphism may be more involved in serotonin and norepinephrine functioning (i.e., lower degradation, higher serotonin/norepinephrine) than in dopamine functioning (i.e., lower degradation, higher dopamine). This involvement in serotonin and norepinephrine functioning, might explain why the originally detected polygenic—moderation effect (chapter 6) was specifically caused by the subset of enzyme-related genes (chapter 7).

Our findings did by no means cover all dopaminergic brain processes and thus other dopaminergic genes that are yet neglected merit consideration, including the Dopamine Receptor D1/2/3 (DRD1, DRD2, DRD3) (Dichter et al., 2012), DOPA decarboxylase (DCC) (Ma et al., 2005), Ankyrin repeat and kinase domain containing 1 (ANK1) (Ponce et al., 2009), Vesicular monoamine transporter 1/2 (VMAT1, VMAT2), Brain Derived Neurotropic Factor (BDNF) (Berton et al., 2006), Dopamine beta-hydroxylase (DBH) (Barkley, Smith, Fischer, & Navia, 2006).

**Reward processing beyond dopamine**

Other neuromodulators than the dopamine ones may also modulate children’s differential responsiveness to rewarding stimuli. This complex interaction of the dopaminergic system with other neuromodulators is not yet fully elucidated and needs further investigation (Moore & Depue, 2016). However, it may very well be that specific neuromodulators, like oxytocin, β-endorphin, and testosterone elicit or inhibit dopaminergic signaling in response to reward. Oxytocin is a neuropeptide that—beyond its involvement in endocrine function—modulates responses to prosocial behavior, like positive parenting behavior and social bonding (Carter, 2014). Indeed, a recent review on the effects of oxytocin administration revealed enhanced social cognitions in encoding and remembering socially relevant emotional formation (Graustella & MacLeod, 2012). β-endorphin is an endogenous opioid peptide that modulates, among others, the experience of rewards and reward-based learning. A review on the relation between β-endorphin and drugs-induced reward and reinforcement, illustrated an important mediating role of β-endorphin in reward processing of alcohol/cocaine (Roth-Deri, Green-Sadan, & Yadid, 2008). Less is known about the neurosteroid testosterone that modulates sensation-seeking, sensitivity to reward, and motivation to act (Bos, Panksepp, Bluthe, & Honk, 2012; Eisenegger, Haushofer, & Fehr, 2011). However, neuroimaging studies demonstrated that testosterone administration acts on reward-seeking processes and enhanced reward dependency (e.g., Van Honk et al., 2011). As such, research combining molecular genetics, neuroimaging, and endocrinology is required to investigate variants in dopaminergic genes and in hormone levels that presumably modulate overall reward processing (Caldu & Dreher, 2007). Ultimately, we need to join forces with other disciplines to enable the investigation of “brain reward-processes-×-environment interactions” more broadly defined (chapter 4).

**Neurotransmitters that modulate general neural activity**

Besides neuromodulators that elicit/inhibit dopaminergic signaling in response to reward, there are ones that modulate neural activity in any type of environmental condition. These neuromodulators might affect children’s sensitivity to environmental context in a general manner. Moore and Depue (2016) refer to this as a “neural constraint” thereby implying that increases (hyperactivity) or decreases (rigidity) in overall neural activity would result in, respectively, a lower or higher threshold value to rewarding stimuli. Hyperactivity relates to easy elicitation of attention by stimuli and the ability to discriminate relevant from irrelevant stimuli, whereas rigidity relates to difficult elicitation of attention, a vigilant state, and difficulties in discriminating relevant from irrelevant stimuli. As such, children with less dopaminergic signaling may have a higher threshold value to be sensitive to reward-based parenting, but this threshold may raise or lower depending on general neural activity. Consider in this regard, Glutamate and GABA are the most plentiful neurotransmitters in the brain and modulate many processes. Glutamate is an excitatory neurotransmitter and GABA an inhibitory neurotransmitter that work together to balance stimulating and tranquilizing overall neural activity in the brain (Petroot, 2002). Serotonin is another inhibitory neurotransmitter that plays a role in many brain processes and modulates overall incoming emotionally-related stimuli (Cools, Roberts, & Robbins, 2008). Norepinephrine is an excitatory neurotransmitter that modulates the overall valance of incoming stimuli, alertness, and attentional focus (Kraevets, Reyes, Unterwald, & Van Bockstaele, 2015). Conceivably, these neurotransmitters—as which we only listed a few—modulate overall neural activity and might relate to hyperactivity or rigidity in dopamine signaling. In fact, this overall neural activity might have caused the MAOA polymorphism to interact with increased sensitivity to parenting in predicting children’s externalizing behavior (chapter 6), irrespective of its direct link with more dopamine signaling (chapter 7).

**Developmental perspective on reward sensitivity**

Molecular genetic studies illustrate that environmental experiences can modify the expression of genes that in turn influence neural reactivity to environmental experiences in the future (Mitchell et al., 2013; Szlyf et al., 2007). DNA methylation differences across the DRD4 and MAOA gene were found in identical twins, suggesting that environmental experiences modified the expression of genes (Wang et al., 2010). This indicates that epigenetic processes might change dopaminergic
gene expression, linked to changes in reward sensitivity. Although, not directly related to underlying epigenetic processes, it has also been found that high levels of testosterone during fetal development are associated with heightened neural reward sensitivity in 8-to-11-years-old children (Lombardo et al., 2012). In addition, children growing up in an adverse environment (e.g., poverty) developed a preference for immediate rather than long-term rewards (Griskevicius, Tybur, Delton, & Robertson, 2011). Moreover, heroin self-administration increased reward sensitivity in nondependent rats, but gradually decreased reward sensitivity in dependent rats (Kenny, Chen, Kitamura, Markou, & Koob, 2006). Thus, it might be that children's reward sensitivity to environmental experiences is not a static "trait" but rather a dynamic feature, fluctuating across developmental phases and experiences. Different reward experiences might mold children's sensitivities to subsequent environmental circumstances, resulting in different patterns of environmental sensitivity over time. Therefore, reward sensitivity could be seen as a continuous state-like, rather than as a categorical and static susceptibility factor, in that some children may be more malleable than others depending on their current degree of dopaminergic functioning and chains of environmental experiences they had before.

**Recommendations for further research and clinical implications**

The central finding in this thesis is that some children, based on genetic variations, benefited more from intervention efforts than others (chaptes 6 and 7), but this finding may also raise questions about clinical implications (chapter 4). Some scholars predict that genetic testing for specific polymorphisms will become increasingly important as a guide to prevention and clinical management (Burke et al., 2002; Van Goozen & Fairchild, 2008). Indeed, G × I research might help to differentiate forms of sensitivity to parenting behavior that are important to tailor personalized intervention and to boost the currently modest effectiveness of interventions. However, there is ample reason to doubt that the relations between genetic markers and intervention effectiveness in the psychological domain can be straightforward enough to inform treatment decisions for individual clients. We illustrated that the surge of interest in dopamine×environment interactions raises concerns about the complex interrelations between different brain systems that are currently not fully understood. For one, reward sensitivity might be domain specific and be fluctuating across development. Without exact knowledge about genetic susceptibility across domains and developmental phases, genetic screening for inclusion in interventions, for instance, would lead to too many false decisions (Ross, Saal, David, Anderson, & American Academy Pediatrics, 2013). Ultimately, we need to join forces with other disciplines to enable the investigation of "brain reward-processes×environment interactions" more broadly defined. GWAS studies to find novel dopaminergic polymorphisms, neuroimaging studies to investigate the dopaminergic underlying mechanisms in the brain, molecular genetics and endocrinology studies to investigate the complex interactions with other neurotransmitters and hormones, epigenetic studies to investigate environmental experiences modifying the expression of (dopaminergic) genes, and social and behavioral studies to investigate the environmental factors and underlying cognitive and behavioral mechanisms that influence child behavior. Until then, an important consideration is to examine behavioral rather than genetic markers, like "behavioral reward-processes×environment interactions".

**Conclusion**

In this thesis, we sought to clarify G × E interactions in child externalizing behavior based on multiple genes influencing the dopamine system. In a longitudinal study, we found that that low parental support longitudinally predicted delinquent behavior, in a diathesis-stress-related manner, but only for those carrying the DRD2 A2 allele. In an experimental intervention study, we found that a dopaminergic polygenic index (DRD2 A1, DRD4 7-repeat, DAT1 10-repeat, MAOA low-activity, COMT val) moderated the intervention efficacy of the IY parent program, in a vantage-sensitivity-related manner. Specifically, boys carrying many dopaminergic polymorphisms decreased significantly in externalizing behavior as a result of their parents' involvement in the IY intervention. In a follow-up study, we found that this detected polygenic-moderation effect was specifically caused by the subset of enzyme genes (COMT, MAOA) rather than by the subset of receptor genes (DRD2, DRD4) or subset of transporter genes (DAT1). Taken together, the findings chronicled in this thesis demonstrated that the dopaminergic system can be considered to be an important neurobiological system that might explain the association between children's sensitivity to parenting behavior and thereby the development of externalizing behavior. However, because the dopaminergic polymorphisms impinge on multiple other neurotransmitters, more research is needed to better understand specific endophenotypic processes that could explain how the complex dopamine network made some children seemingly more sensitive to parenting behavior than others.


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Elevated levels of externalizing behavior in the early years of life might forecast a variety of problems later in childhood. Left untreated, externalizing behavior often worsens with age and might develop into persistent patterns of severe externalizing behavior. These patterns generate substantial social and economic costs to families and society and underscore the need to learn more about the causes of externalizing behavior in childhood. Negative parenting behavior is considered to be one of the strongest potentially modifiable risk factors that contributes to the development of child externalizing behavior. Recent research suggests that the likelihood to develop externalizing behavior as a consequence of negative parenting behavior, depends in part on children’s genetic make-up (i.e., G × E). However, G × E findings have raised criticism and serious concerns regarding mixed findings and replications, making it difficult to draw conclusions. One important challenge is to fill in the details in neurobiological processes that link genes and environment to child externalizing behavior. The dopaminergic system is considered to be such a neurobiological component because of its link with reward-based learning and reward sensitivity. Another challenge pertains to G × E confounders. Many G × E studies used correlational designs that do not permit causal inferences and are unable to rule out alternative interpretations, like passive gene-environment correlations (rGE). Experimental studies can overcome these concerns by design. The aim of this thesis was to clarify G × E interactions in child externalizing behavior based on multiple genetic polymorphisms influencing the dopamine system.

In chapter 2, we conducted a longitudinal study to predict G × E in severe externalizing behavior over time. More specifically, we investigated whether negative parenting behavior (high psychological control and low support) longitudinally predicted the development of delinquent behavior and whether the likelihood to develop such behavior, as a consequence of negative parenting behavior, depended in part on genetic variation in two dopamine-related genes (DRD2, DRD4). In this study, we controlled for passive genetic effects (i.e., parents’ genotype, Parents’ Genotype × Adolescents’ Genotype, and Parents’ Genotype × Parenting). Data (N = 308; Mage = 13.4, SD = 51; 47% boys) were derived from the 5-wave longitudinal Dutch survey study Family and Health, which investigates family processes in relation to various health behaviors in adolescence. Main effects of parenting behavior illustrated that adolescents showed higher levels of delinquent behavior at baseline in response to low parental support, and relatively high but decreasing delinquency across late adolescence in response to high psychological control. In addition to these main effects of parenting behavior, we found evidence for G × E interactions. More specifically, the DRD2 A2A2 genotype interacted with low parental support. This interaction was curvilinear; in that adolescents carrying the A2A2 genotype showed steeper increases in delinquent behavior across early to mid-adolescence followed by quicker decreases across late adolescence in response to low parental support. The DRD4 7-repeat allele interacted with high psychological control – however, when accounting for passive rGE, this interaction was no longer significant. In addition, no significant interactions emerged for DRD4 × Parental Support nor for DRD2 × Psychological Control.
Second, an intervention study was carried out in which the parenting environment was experimentally manipulated (chapters 3-7). In this study, we again considered negative parenting behavior to be a risk factor that contributes to the development of externalizing behavior. Data ($N = 387$, $M = 6.21$, $SD = 1.33$, 55.3 % boys) were derived from the ORCHIDS study (Observational Randomized Controlled Trial on Childhood Differential Susceptibility) conducted in The Netherlands. The ORCHIDS study addresses differential effectiveness—across children with varying genetic characteristics—of the Incredible Years (IY) parent training in reducing externalizing behavior in 4-8-year-old children showing moderate-to-high levels of such problems by enhancing a warm parent-child relationship through child-directed play, praise and rewards, social and emotional coaching, and effective limit setting and handling (e.g., ignoring and time-out techniques).

In chapter 3, we described the study protocol for project ORCHIDS and delineated the hypotheses about genetic moderation of intervention efficacy. However, G × E research—is fraught with difficulties and raises several serious ethical questions. In chapter 4, we made some of these ethical questions explicit, discussing specifically whether it is ethically responsible to withhold an effective treatment, to what extent or under which circumstances genetic data should be disclosed; whether researchers should be allowed to collect genes of both children and parents; and what costs and benefits of personalized interventions are based on genetic screening.

In chapter 5, we investigated the effectiveness of the IY parent program and effects of previously suggested sociodemographic and intervention-based moderator variables (i.e., initial severity of externalizing behavior, child gender, social economic status, family composition, number of sessions parents attended). Using full intention-to-treat analyses, questionnaire and observation data from 387 parents and children ($M = 6.2$, $SD = 1.33$, 55.3 % boys) across pretest, posttest, and follow-up were used, correcting for multiple testing. IY proved successful in decreasing parent-reported child externalizing behavior, decreasing parent-reported negative parenting behavior, and increasing parent-reported and observed positive parenting behavior. No intervention effects were found for observed child externalizing behavior, observed negative parenting behavior, and parent-reported and observed child prosocial behavior. Out of 40 tested moderation effects (i.e., eight outcomes times five moderators) only three significant moderation effects appeared, indicating that no systematic evidence emerged for moderation of IY effects by these factors.

In chapter 6, we investigated whether children ($N = 341$) scoring higher on a polygenic plasticity index, based on 5 dopaminergic genes (DRD4, DRD2, DAT1, MAOA, COMT), benefited the most from the IY parent program. IY proved most effective in decreasing parent-reported (but not observed) externalizing behavior in boys (but not girls) carrying more than fewer dopaminergic polymorphisms. This gene × intervention effect was most pronounced in the case of boys whose parents manifested the most positive change in parenting in response to the intervention. Moreover, results proved robust across a variety of sampling specifications (e.g., intention-to-treat, ethnicity). Thus, we showed that some children were more sensitive to intervention-induced positive parenting than others based on genetic characteristics related to the dopaminergic system.

In chapter 7, we built on results of our prior report documenting genetic moderation of intervention efficacy in the case of boys ($N = 190$). In this study, we sought to gain insight into which of the polymorphisms related to three dopaminergic subsystems—receptor (DRD4, DRD2), transport (DAT1) and/or enzyme (MAOA, COMT)—might be principally responsible for the moderation effect detected in chapter 6. Latent growth modeling revealed that the originally detected polygenic—moderation effect was specifically caused by the subset of enzyme genes. Boys carrying more enzyme-related polymorphisms (but not boys carrying fewer of such polymorphisms) decreased significantly in externalizing behavior as a result of their parents’ involvement in the IY intervention. This effect remained even when we controlled for other dopaminergic subsets.

The overall results chronicled in this thesis demonstrated that the dopamine system can be considered to be an important underlying neurobiological system in explaining the association between children’s sensitivity to, respectively, positive and negative parenting behavior. Notably, we illustrated that complex behavioral traits, like externalizing behavior, are likely to be influenced by multiple rather than single dopaminergic genes. However, we also found mixed findings regarding the genetic variants (DRD2 A2 allele vs. A1 allele) contributing to externalizing behavior, making it difficult to investigate cumulative effects of dopaminergic genes. Moreover, the hypothesized G × E interactions were not systematically found across the models tested, emphasizing that replication is essential to confirm the present evidence. Also, different patterns of G × E interactions were found. Rather than finding differential susceptibility, we found a diathesis-stress and a vantage sensitivity type interaction. Although this remains speculation, perhaps a differential susceptibility type interaction would have been obtained if externalizing behavior was measured along a continuum from dysfunction to competence rather than from dysfunction to its absence (chapter 2) or within a clinical sample that included more severe externalizing behavior and/or more severity in environment context (chapters 6 and 7). In addition, we included multiple genes influencing the dopamine system, but did not investigate complex interrelations of the dopamine system with other neurotransmitter systems, other reward-related neurotransmitters, and other less well-known dopaminergic genes.

Thus, although the present thesis has made an important contribution to the field of G × E research by focusing on a specific underlying neurobiological system and conducting a longitudinal and experimental study, more research is needed to better understand specific endophenotypic processes that could explain how the complex dopamine network made some children seemingly more sensitive to parenting behavior than others.
Een verhoogd niveau van externaliserend probleemgedrag bij jonge kinderen kan leiden tot vele problemen op latere leeftijd. Wanneer niet vroegtijdig ingegrepen wordt, kunnen deze gedragsproblemen toenemen met leeftijd en leiden tot hardnekkige vormen van ernstig externaliserend probleemgedrag. Deze hardnekkige vormen van dergelijk gedrag zorgen voor grote sociale en economische kosten voor gezin en samenleving en onderstreept het belang van onderzoek naar de oorzaak hiervan. Negatief opvoedingsgedrag wordt gezien als één van de sterkste potentieel veranderbare risicofactoren die bijdraagt aan de ontwikkeling van externaliserend probleemgedrag. Recent onderzoek laat zien dat sommige kinderen op basis van hun genetische opmaak gevoeliger kunnen zijn voor negatief opvoedingsgedrag en daardoor eerder de kans hebben op het ontwikkelen van externaliserend probleemgedrag. Er is echter veel kritiek op de resultaten van onderzoek naar de interactie tussen genen en omgeving (G × E). Resultaten van G × E studies zijn vaak tegenstrijdig en moeilijk te repliceren, waardoor het trekken van een eenduidige conclusie moeilijk is. Een belangrijke uitdaging is het zicht krijgen op onderliggende neurobiologische processen die genen en omgeving koppelen aan externaliserend probleemgedrag. Het dopaminesysteem wordt gezien als een belangrijk neurobiologisch component, omdat dopamine verbonden is aan beloningsgevoeligheid en beloningsgericht leren. Een andere uitdaging heeft te maken met variabelen die de interpretatie van G × E resultaten kunnen verstoren. Veel G × E studies gebruiken een correlatieve onderzoekskop, waarbij het niet mogelijk is om causale gevolgtrekkingen te onderzoeken, hierdoor kunnen alternatieve interpretaties, zoals passieve gen-omgeving correlatie (rGE), niet helemaal uitgesloten worden. Experimentele studies kunnen deze problemen met het interpreteren van G × E bevindingen ondervangen. Het doel van dit proefschrift was om G × E interacties met betrekking tot externaliserend probleemgedrag te verduidelijken, waarbij specifiek gekeken is naar verschillende genetische polymorfismen die het dopaminesysteem beïnvloeden.

In hoofdstuk 2 hebben we een longitudinale studie uitgevoerd om te onderzoeken of G × E interacties de ontwikkeling van ernstig externaliserend probleemgedrag kunnen voorspellen. Specifiek, hebben we onderzocht of negatief opvoedingsgedrag (hoge psychologische controle en lage ouderlijke steun) de ontwikkeling van delinquent gedrag voorspelt. Daarnaast hebben we onderzocht of dit effect van negatief opvoedingsgedrag op delinquent gedrag afhankelijk was van genetische variatie in twee dopamine gerelateerde genen (DRD2, DRD4). In deze studie hebben we gecontroleerd voor passieve genetische effecten (genotype ouders, Genotype Ouder × Genotype Adolescenten en Genotype Ouder × Opvoeding). Data (N = 308, Mage = 13.4, SD = 5.1, 47% jongens) werden gebruikt van de Nederlandse Familie en Gezondheid studie die bestaat uit 5-jaarlijkse meetrondes. Deze dataset richt zich op familieprocessen die verschillende gezondheidsgeraden in de adolescentie kunnen beïnvloeden. Als hoofdffect van opvoeding vonden we dat adolescenten meer delinquent gedrag (bij aanvang van de studie) lieten zien in reactie op lage ouderlijke steun. In reactie op hoge psychologische controle, lieten adolescenten een relatief hoge maar vooral een sterke afname in delinquent gedrag zien gedurende late
adolescentie. Naast deze hoofdeffecten van opvoeding vonden we bewijs voor \( G \times E \) interacties. Specifiek vonden we een interactie tussen het \( \text{DRD2 A2A2 genotype en lage ouderlijke steun.} \) De interactie was curvilinear, dat betekent dat adolescenten met het A2A2 genotype een grotere toename in delinquent gedrag lieten zien in vroge tot midden adolescentie gevolgd door een snellere afname in late adolescentie, maar alleen in reactie op lage ouderlijke steun. Ook vonden we een interactie tussen het \( \text{DRD4} \times \text{repeat allel en hoge psychologische controle} \) – echter, wanneer we controleerden voor passieve \( rG \), vonden we dat de interactie niet langer significant was. Er werden geen significante interacties voor \( \text{DRD4} \times \text{Ouderlijke Steun en DRD2} \times \text{Psychologische Controle} \) gevonden.

Daarna werd er een interventiestudie uitgevoerd waarbij de opvoedingsomgeving experimenteel gemanipuleerd was (hoofdstukken 3-7). In deze studie hebben we weer gekeken of negatief opvoedingsgedrag een risicofactor was die bijdroeg aan de ontwikkeling van externaliserend probleemgedrag, maar ook of het één van de sterkste potentieel veranderbare factoren was. Data (\( N = 387 \), Mage = 6.21, SD = 1.33, 55.3% jongens) werden gebruikt van de ORCHIDS studie (Observational Randomized Controlled Trial on Childhood Differential Susceptibility) uitgevoerd in Nederland. De ORCHIDS studie richt zich op de vraag of sommige kinderen op basis van specifieke genetische eigenschappen meer gevoelig zijn voor de oudercursus Incredible Years \( \times \text{Psychologische Controle} \)

In hoofdstuk 3 hebben we het studieprotocol voor het ORCHIDS project beschreven en hebben we de hypotheses/verwachtingen over de genetische moderalte van het interventie-effect afgebakend. Echter, het gebruik van genetische data in interventie studies is beladen en kan leiden tot ethische vragen. In hoofdstuk 4, hebben we een aantal ethische vragen expliciet gemaakt, waarbij specifiek besproken werd in hoeverre het ethisch verantwoord was om effectieve behandeling te onthouden, in welke mate en onder welke omstandigheden genetische gegevens moeten worden verstrekt aan ouders en kinderen; of onderzoekers genen van zowel kinderen als ouders zouden mogen verzamelen; en wat de kosten en lasten zijn van eventuele gepersonaliseerde interventies op basis van een (genetische) screening.

In hoofdstuk 5 hebben we onderzoek gedaan naar de effectiviteit van de \( \text{IY-oudercursus.} \) Ook hebben we gekeken of de effectiviteit van \( \text{IY} \) beïnvloed werd door uit eerder onderzoek gesuggererde sociaal-demografische en interventie-gebaseerde moderatoren (initiële ernst van de gedragsproblemen, geslacht kind, sociaaleconomische status, gezins samenstelling en het aantal sessies die ouders in de interventie hebben bijgewoond). Hiervoor hebben we gebruik gemaakt van een volledige “intention-to-treat-analyse”, vragenlijsten en observatiegegevens van 387 ouders en kinderen (Mage = 6.21, SD = 1.33, 55.3% jongens). Deze data werden verzameld op een voormeting, nameting en follow-up. In deze studie hebben we gecorrigeerd voor de meerdere toetsen die we hebben uitgevoerd. \( \text{IY} \) bleek succesvol in het verminderen van ouder-gerapporteerd externaliserend probleemgedrag, het verminderen van ouder-gerapporteerd negatief opvoedingsgedrag en het toenemen van ouder-gerapporteerd en geobserveerd positief opvoedingsgedrag. Er werden geen interventie-effecten gevonden voor geobserveerd externaliserend probleemgedrag; geobserveerd negatief opvoedingsgedrag en ouder-gerapporteerd en geobserveerd prosociaal gedrag in kinderen. Van de 40 moderatie-effecten die we getoetst hebben (acht uitkomsten keer vijf moderatoren) waren slechts drie effecten significant, dit betekent dat er geen systematisch bewijs werd gevonden waaruit blijkt dat bovengenoemde moderatoren de IY-effecten daadwerkelijk beïnvloeden.

In hoofdstuk 6 hebben we onderzocht of kinderen (\( N = 341 \)) die een hogere score hadden op een polygene plasticiteitsindex, gebaseerd op 5 dopaminegenen (\( \text{DRD4}, \text{DRD2}, \text{DAT1}, \text{MAOA}, \text{COMT} \)), het meest profiterden van het \( \text{IY-ouderprogramma.} \) \( \text{IY} \) bleek het meest effectief in het verminderen van ouder-gerapporteerd (maar niet geobserveerd) externaliserend probleemgedrag bij jongens (maar niet bij meisjes) die meer in plaats van minder dopamynerge polymorfismen droegen. Het gen-gen-interventie effect was het meest uitgesproken in het geval van jongens waarvan de ouders de meeste positieve verandering in opvoedingsgedrag lieten zien in reactie op de interventie. Bovendien bleken deze resultaten robuust in verschillende steekproef specificaties (“intention-to-treat-analysis”, etnicieteit). Met andere woorden, we hebben aangetoond dat sommige kinderen gevoeliger zijn voor de door de interventie veraarzakte positieve verandering in opvoedingsgedrag, omdat zij specifieke genetische kenmerken hebben die verband houden met het dopaminsysteem.

In hoofdstuk 7 zijn we dieper ingegaan op het onderzoek waarin we een genetische moderalte van het interventie-effect in jongens vonden (\( N = 190 \)). In deze studie wilden we te weten komen welke polymorfismen gerelateerd aan drie dopaminerige subsystemen—receptor (\( \text{DRD4}, \text{DRD2} \)), transporteur (\( \text{DAT1} \)) en/of enzym (\( \text{MAOA}, \text{COMT} \))—voornamelijk verantwoordelijk waren voor de genetische moderalte van de interventie-effect in hoofdstuk 6. Een latent groeimodel liet zien dat het oorspronkelijk gevonden polygene-moderatie effect specifiek werd veroorzaakt door de subset van enzymgenen. Jongens met meer enzym-geregelateerde polymorfismen (maar niet jongens met minder dergelijke polymorfismen) lieten een significante afname zien in externaliserend probleemgedrag als gevolg van hun ouders’ deelname aan de IY-interventie. Dit effect bleef zelfs significant toen we controleerden voor de andere dopamine subsets.

De algehele resultaten in dit proefschrift lieten zien dat het dopaminsysteem kan worden beschouwd als een belangrijk onderliggend neurobiologisch systeem dat het verband tussen de gevoeligheid van kinderen voor, respectievelijk, positief en negatief
opvoedingsgedrag kan verklaren. Met name hebben we aangetoond dat complexe
gedragskenmerken, zoals externaliserend probleemgedrag, kunnen worden beïnvloed
door de samenhang van meerdere-, in plaats van enkele, dopaminegenen. Toch vonden
we ook tegenstrijdige bevindingen met betrekking tot genetische varianten (DRD2
A2 allele vs. A1 allele) die bijdroegen aan externaliserend probleemgedrag, waardoor
het moeilijk was om cumulatieve effecten van de dopaminegenen te onderzoeken.
Bovendien werden de veronderstelde G × E interacties niet systematisch gevonden
voor de modellen die we getest hebben, wat benadrukt dat replicatie belangrijk is
om huidige onderzoekresultaten te bevestigen. Ook vonden we verschillende vormen
van G × E interacties. In plaats van het vinden van een 'differential susceptibility' type
interactie, vonden we een diathese-stress en een 'vantage sensitivity' type interactie.
Hoewel het speculeren blijft, zou het kunnen dat we een 'differential susceptibility'
type interactie hadden kunnen vinden als externaliserend probleemgedrag gemeten
was langs een schaal van dysfunctie tot competentie in plaats van dysfunctie en de
afwezigheid hiervan (hoofdstuk 2). Daarnaast hadden we wellicht een dergelijk type
interactie kunnen vinden als we een klinische steekproef met meer ernstige gedrag-
sproblemen hadden gehad of er meer ernst in de opvoedingsomgeving had gezeten
(hoofdstuk 6 en 7). Tevens hebben we de invloed van meerdere genen gerelateerd
aan het dopaminesysteem onderzocht, maar niet gekeken naar complexe onderlinge
verbanden van het dopaminesysteem met andere neurotransmittersystemen, andere
beloning gerelateerde neurotransmitters en andere minder bekende dopaminegenen.
Samengevat, hoewel dit proefschrift een belangrijke bijdrage heeft geleverd op het
gebied van G × E onderzoek door het zich te richten op een specifiek onderliggend
neurologisch systeem en het uitvoeren van longitudinaal en experimenteel onderzoek,
is er meer onderzoek nodig naar specifieke endofenotypische processen die kunnen
verklaren hoe het complexe dopaminenetwork  ervoor zorgt dat sommige kinderen
schijnbaar gevoeliger zijn voor opvoeding dan anderen.


¹ The first two authors contributed equally to this work
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Rabia is nu werkzaam als opleidingscoördinator van de Research Master Behavioural Science van de Radboud Universiteit Nijmegen. Daarnaast werkt zij, samen met collega’s van de Universiteit van Amsterdam en de Radboud Universiteit, aan een onderzoeksvoorstel met als doel om haar onderzoek op het gebied van interventie en gedragsproblematiek bij kinderen voor te zetten.